(22)

No. 04-607

DEC 2 [illegible]

IN THE

# Supreme Court of the United States

LABORATORY CORPORATION OF AMERICA HOLDINGS (doing business as LabCorp),

*Petitioner*,

v.

METABOLITE LABORATORIES, INC. and COMPETITIVE TECHNOLOGIES, INC.,

*Respondents.*

**On Writ of Certiorari to the United States Court of Appeals for the Federal Circuit**

## JOINT APPENDIX
## (Volume II of II)


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**PETITION FOR CERTIORARI FILED NOVEMBER 3, 2004**
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**Plaintiffs' Exhibit Demo. 01**

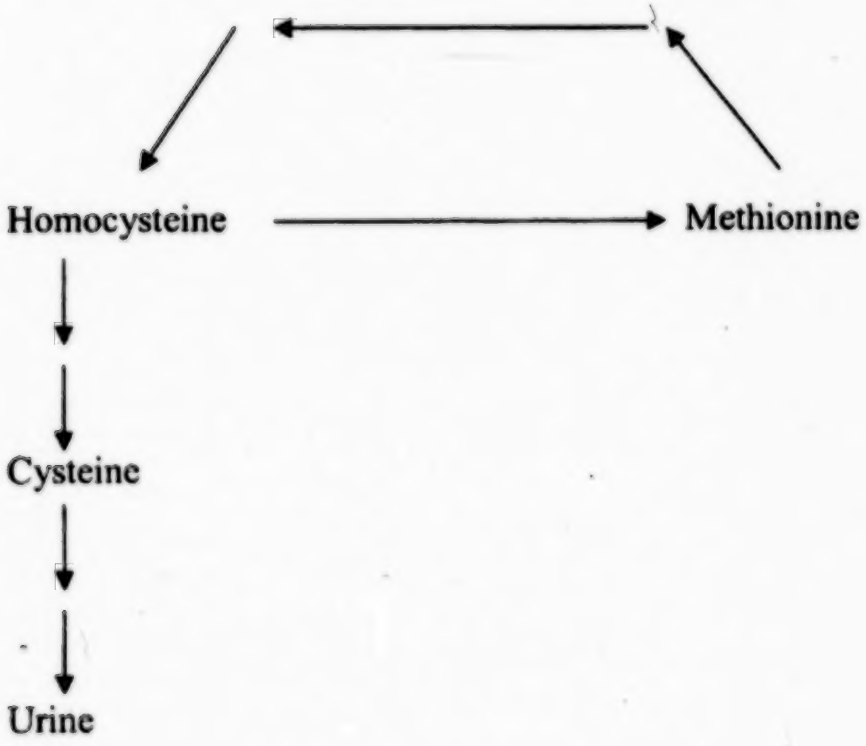
## DESIRABLE TRAITS OF A USEFUL DIAGNOSTIC TEST

1. READILY FOUND – It can readily be found in blood or urine.
2. RELIABLE – It can be measured reliably.
3. STABLE – It is chemically stable over minutes or hours.
4. CONSTANT WHILE HEALTHY – The finding remains relatively constant while the patient is in healthy/normal state.
5. CORRELATES – The marker "correlates" (mutual or reciprocal relationship) with disease state compared with healthy state.
6. MEASURABLE DIFFERENCE FROM HEALTHY – There is always a measurable difference from an individual's healthy/normal range, and often a measurable difference from the population normal range.
7. TREATMENT AVAILABLE – Treatment is available that brings the test finding back toward that individual's healthy/normal range.
8. PREDICTS OUTCOME – When suggested treatment is applied, patient's healthy/normal status returns, or further deterioration is prevented.
9. EARLY WARNING SYSTEM – Marker detection indicates the diseased or impaired state in time to interrupt or reverse early physical/mental manifestations.

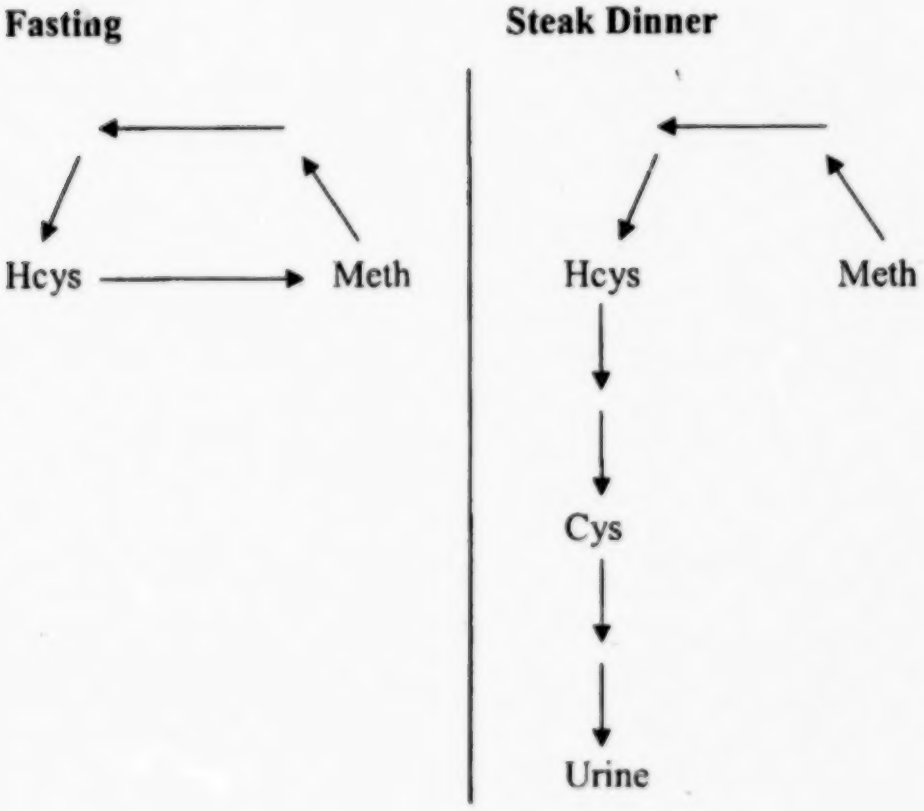
* * * *

**Plaintiffs' Exhibit Demo. 02**

***Methionine Pathway***



**Plaintiffs' Exhibit Demo. 03**



**Plaintiffs' Exhibit Demo. 04**

## *Key Amino Acids in Methionine Pathway*

| | Meth | Hcys | Cys |
|---|---|---|---|
| Building block for proteins | ☑ | | ☑ |
| Needed for genes | ☑ | | |
| Needed for > 100 different processes | ☑ | | |

**Plaintiffs' Exhibit Demo. 05**

## *Possible* Metabolic Changes

| | Meth | Hcys | Cys |
|---|---|---|---|
| #1 Hcys → ($B_{12}$+F) → Meth | — | — | — |
| #2 Hcys → ($B_{12}$+F) → Meth | ↓ | ↑ | — |

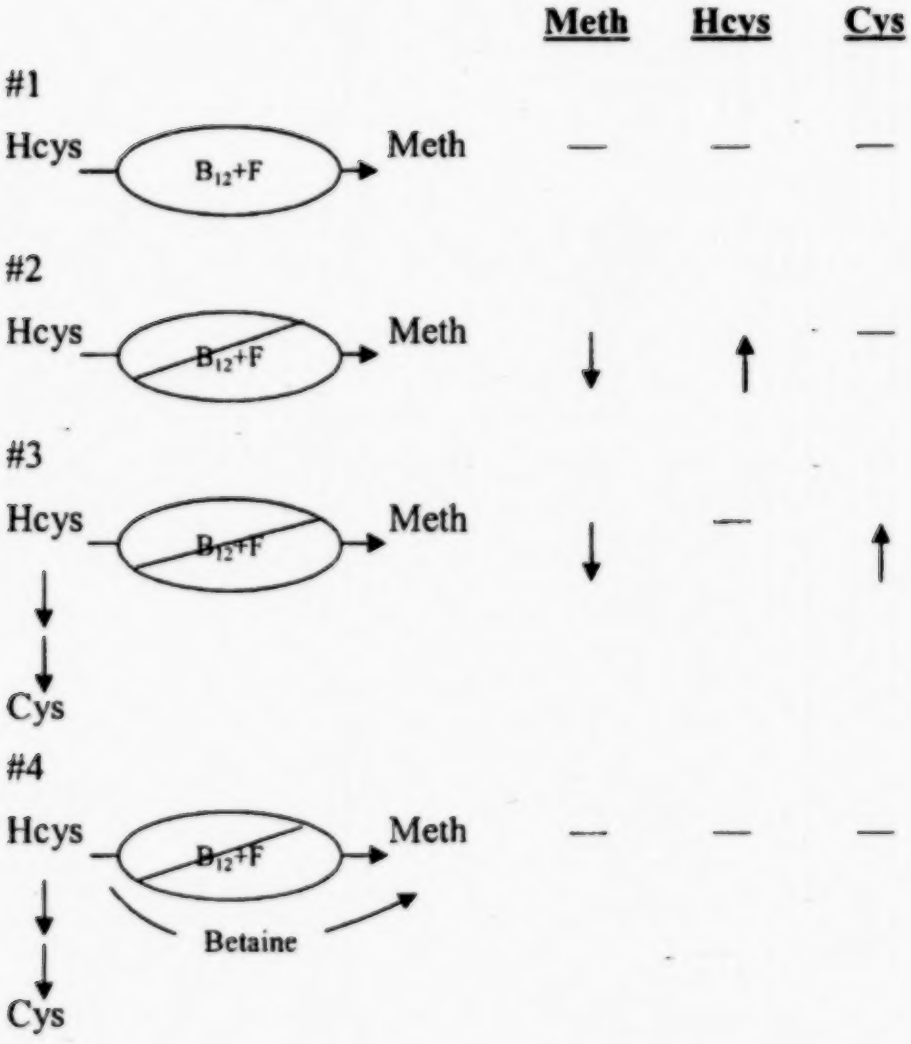
**Plaintiffs' Exhibit Demo. 06**

## ***Possible*** Metabolic Changes

| | Meth | Hcys | Cys |
|---|---|---|---|
| #1 Hcys → ($B_{12}$+F) → Meth | — | — | — |
| #2 Hcys → ~~($B_{12}$+F)~~ → Meth | ↓ | ↑ | — |
| #3 Hcys → ~~($B_{12}$+F)~~ → Meth; Hcys ↓ Cys | ↓ | — | ↑ |

**Plaintiffs' Exhibit Demo. 07**

## ***Possible*** **Metabolic Changes**



**Plaintiffs' Exhibit Demo. 08**

## Three Basic Mechanisms that Cause Deficiencies in $B_{12}$ and Folate

| | Mechanism | | |
|---|---|---|---|
| **Condition** | **Poor Diet** | **Poor Absorption** | **Vitamin Defect** |
| 1. Age | ☑ | ☑ | |
| 2. Gender | ☑ | | |
| 3. Smoking | ☑ | | ☑ |
| 4. Alcohol | ☑ | ☑ | ☑ |
| 5. Medications | | ☑ | ☑ |
| 6. Kidney Disease | ☑ | ☑ | ☑ |
| 7. Thyroid Disease | | ☑ | ☑ |

**Plaintiffs' Exhibit Demo. 09**

## Use of Assays for Total Homocysteine to Detect Various Common Conditions

| Condition | Used for Detection |
|---|---|
| 1. Age | **NO** |
| 2. Gender | **NO** |
| 3. Smoking | **NO** |
| 4. Alcohol | **NO** |
| 5. Medications | **NO** |
| 6. Kidney Disease | **NO** |
| 7. Thyroid Disease | **NO** |
| 8. $B_{12}$ Deficiency | **YES** |
| 9. Folate Deficiency | **YES** |

**Plaintiffs' Exhibit Demo. 10**

## Select Manifestations of $B_{12}$ and Folate Deficiency

| Manifestations | Treatment if Total Homocysteine is Elevated |
|---|---|
| Big red blood cells | **$B_{12}$/Folate** |
| Not enough red blood cells | **$B_{12}$/Folate** |
| Abnormal bone marrow | **$B_{12}$/Folate** |
| Not enough white blood cells | **$B_{12}$/Folate** |
| Sore feet and hands | **$B_{12}$/Folate** |
| Poor memory | **$B_{12}$/Folate** |
| Difficulty walking | **$B_{12}$/Folate** |
| Poor control of urination | **$B_{12}$/Folate** |
| Poor control of bowel movements | **$B_{12}$/Folate** |
| Neural tube defects | **$B_{12}$/Folate** |
| Heart attacks | **$B_{12}$/Folate** |
| Strokes | **$B_{12}$/Folate** |

**Plaintiffs' Exhibit Demo. 16**

## What Abbott Laboratories Teaches About Homocysteine in Its *Home Study Kit* Article *"Homocysteine: The New Risk Factor – Introduction to Patient Management"*

(© 1999)
(L000561 - L000607)

"This CME [Homestudy] activity is sponsored in part by [a Division of] Abbott Laboratories" (L000561)

"Target audience: physicians in private practices and health-care maintenance organizations, across all geographic areas, who evaluate and treat patients for coronary risk factors (eg, cardiology, internal medicine, family practice, general practice)" (L000565)

"Several vitamins function as cofactors and substrates in methionine and homocysteine metabolism, including folate, vitamin $B_6$, and vitamin $B_{12}$. A number of studies have demonstrated inverse relationships between homocysteine concentrations and levels of both folic acid and B vitamins, and these have recently been reviewed.[4]" (L000565)

"Other studies have found that vitamin supplementation can normalize homocysteine concentrations in most individuals." (L000565)

## What Abbott Laboratories Teaches About Homocysteine in Its *Home Study Kit* Article *"Homocysteine: The New Risk Factor – Introduction to Patient Management"* *(cont.)*

"META-ANALYSIS OF HOMOCYSTEINE-LOWERING STUDIES. A recent meta-analysis summarized the results of 12 randomized, placebo-controlled trials in over 1,100 subjects to compare the homocysteine-lowering effects of folic acid alone or in combination with vitamin $B_6$ or vitamin $B_{12}$.[88]" (L000591)

"[T]he meta-analysis found that the use of 0.5 to 5 mg dietary folic acid daily reduced homocysteine concentrations by 25% ($P$ <0.001). The addition of 0.5 mg vitamin $B_{12}$ daily reduced the homocysteine levels by another 3% to 10% over the reduction with folic acid alone. However, the addition of 16.5 mg vitamin $B_6$ daily produced no significant supplemental reducing effect on blood homocysteine levels." (L000591)

**Plaintiffs' Exhibit Demo. 18**

## What LabCorp's [Directory of Services] Teaches Doctors About Megaloblastic Anemia (online pp. 1-2)

Anemia Profile, Megaloblastic, Serum (706960)

"Serum methylmalonic acid and homocysteine are the primary metabolic tests for diagnosing and distinguishing between B12 and folate deficiency."

"This method is a more specific diagnostic tool for B12 and folate deficiencies as it has been shown that some patients with deficiencies will have normal or borderline serum B12 or folate."

"Elevated metabolite levels do not correct with B12, folate, or B6 treatment unless vitamin deficiency coexists."

**Plaintiffs' Exhibit Demo. 19**

## What LabCorp's [Directory of Services] Teaches Doctors About the Homocysteine-Only Test

"Thus, the laboratory diagnosis of cobalamin and folate deficiency will probably account for the largest number of plasma/serum Hcy determinations."

Ueland, et al., *Clinical Chemistry*, 1993
Total homocysteine in Plasma or Serum: Methods and Clinical Applications (at p. 1776)

---

"In this population, folate was the most important determinant of plasma homocyst(e)ine, even in subjects with apparently adequate nutritional status of this vitamin."

Verhoef, et al., *American Journal of Epidemiology*, 1996
Homocysteine Metabolism and Risk of Myocardial Infarction: Relation with Vitamins $B_6$, $B_{12}$ and Folate (at p. 847)

---

"Plasma homocysteine concentrations correlated negatively with both serum vitamin B12 and folate (Table 2). Three studies of patients with peripheral vascular disease and stroke yielded similar results (41, 50, 52)."

Robinson, et al., *Cleveland Clinic Journal of Medicine*, 1994
Homocysteine and Coronary Artery Disease (at p. 444)

---

## What LabCorp's [Directory of Services] Teaches Doctors About the Homocysteine-Only Test

*(cont.)*

"The H(e) concentrations were independent of other risk factors for peripheral artherosclerotic disease, but increased H(e) was observed mainly in subjects with low concentrations of serum folate (47)."

Malinow, *Clinical Chemistry*, 1994
Plasma Homocyst(e)ine and Arterial Occlusive Diseases: A Mini-Review (at p. 175)

---

"The authors conclude that elevated homocyst(e)ine levels in the elderly may, in great part, be attributed to vitamin status."

Fortin and Genest, *Clinical Biochemistry, 1995*
Measurement of Homocyst(e)ine in the Prediction of Arteriosclerosis (at p. 159)

---

"Moderate and intermediate hyperhomocyst(e)inemia is most commonly seen in subjects with low concentrations of serum folate that are due to either inadequate nutrition or to certain medications (40, 66)."

Kang, et al., *Annual Review of Nutrition*, 1992
Hyperhomocyst(e)inemia as a Risk Factor for Occlusive Vascular Disease (at p. 282)

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**Plaintiffs' Exhibit Demo. 20**

## What LabCorp Teaches Doctors About the Treatment of Homocysteine Levels in its [Directory of Services] for the Homocysteine-Only Test

"Total homocysteine in serum/plasma is increased markedly in patients with cobalamin or folate deficiency, and decreases only when they are treated with the deficient vitamin."

Ueland, et al., *Clinical Chemistry*, 1993
Total homocysteine in Plasma or Serum: Methods and Clinical Applications (at p. 1764)

---

"Furthermore, our study supports the view that adequate folate intake from diet or supplements might be an important step toward normalizing homocyst(e)ine levels."

Verhoef, et al., *American Journal of Epidemiology*, 1996
Homocysteine Metabolism and Risk of Myocardial Infarction: Relation with Vitamins $B_6$, $B_{12}$ and Folate (at p. 858)

---

"Vitamin therapy, which lowers the plasma homocysteine concentration, is simple, nontoxic, naturally occurring, and has a wide therapeutic index."

Robinson. et al., *Cleveland Clinic Journal of Medicine*, 1994
Homocysteine and Coronary Artery Disease (at p. 447)

---

## What LabCorp Teaches Doctors About the Treatment of Homocysteine Levels in its [Directory of Services] for the Homocysteine-Only Test *(cont.)*

"The increased concentrations of H(e) are readily corrected by folic acid, occasionally supplemented with pyridoxine, vitamin B12, choline, or betaine."

Malinow. *Clinical Chemistry*, 1994
Plasma Homocyst(e)ine and Arterial Occlusive Diseases: A Mini-Review (at p. 173)

---

"Low-dose folic acid, vitamin B6 or both are used to reduce elevated H(e) levels in affected individuals. Resistant cases may be treated with betaine."

Fortin and Genest, *Clinical Biochemistry*, 1995
Measurement of Homocyst(e)ine in the Prediction of Arteriosclerosis (at p. 155)

---

"Irrespective of the etiology, moderate and intermediate hyperhomocyst(e)inemia is readily correctable by supplementation with folate and/or betaine."

Kang, et al., *Annual Review of Nutrition*, 1992
Hyperhomocyst(e)inemia as a Risk Factor for Occlusive Vascular Disease (at p. 294)

---

**Plaintiffs' Exhibit Demo. 21**

**What LabCorp's [Directory of Services] Teaches Doctors About the Homocysteine-Only Test in its CME Article Entitled, *"Homocyst(e)ine-The Bad Amino Acid?"***
**by Peter W. Wentz, Ph.D.**
**(L001307 – L001312)**

"The most common cause of an elevated basal level of homocyst(e)ine is a deficiency of one of the B complex vitamins, folate in particular." (page 4)

"HOW CAN ELEVATED HOMOCYSTEINE LEVELS BE LOWERED? Vitamin therapy and dietary modification can work together to help lower plasma homocyst(e)ine levels." (page 4)

"Total homocyst(e)ine levels in normal subjects and patients with vascular disease correlate with serum levels of vitamin B12, folate and vitamin B6 and with the oral intake of these B-complex vitamins. Both oral and parenteral administration of these vitamins normalizes elevated homocyste(e)ine levels in virtually all patients with vascular disease, provided that renal insufficiency is not present." (page 4)

## What LabCorp's [Directory of Services] Teaches Doctors About the Homocysteine-Only Test in its CME Article Entitled, *"Homocyst(e)ine-The Bad Amino Acid?"*

*(cont.)*

"The conclusion of the study indicates that all patients with folate levels below 4.4 ng/ml should have a homocyst(e)ine level measured before vitamin therapy is initiated." (page 4)

"Serum metabolite levels can be rechecked five to 15 days after vitamin therapy is initiated." (page 5)

"The most important determinants of tHcy have been identified as age and sex, folate and vitamin B12 status, and renal function. Vitamin supplementation, especially folic acid, is effective at lowering blood tHcy levels." (page 6)

**Plaintiffs' Exhibit Demo. 22**

## What LabCorp's [Directory of Services] Teaches Doctors About The Allen/Stabler/Lindenbaum Patents in its CME Article Entitled *"Megaloblastic Anemia: Laboratory Screening and Confirmation"*

## by Peter W. Wentz, Ph.D. (online)

"The metabolite patterns are characteristic for the particular vitamin involved. These biochemical pathways have been studied extensively and the clinical implications have been described in various publications appearing over the course of more than 30 years.[18-23] Subsequently, the clinical correlations and analytical methodology became the basis for two U.S. patents.[24,25]["] (page 5)

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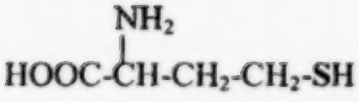
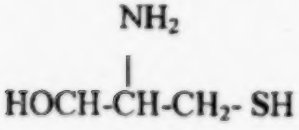
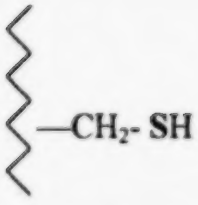
24. **United States Patent Number: 4,940,658**. Issued: July 10, 1990. Invention: Assay for sulfhydryl amino acids and methods for detecting and distinguishing cobalamin and folic acid deficiency. Inventors: Robert H. Allen, [Sally] P. Stabler, and John Lindenbaum. (at page 7 of online text)
25. **United States Patent Number: 5,374,560**. Issued: December 20, 1994. Invention: Method for screening and distinguishing between cobalamin and folic acid deficiency based on assay for cystathionine and 2-methylcitric acid. Inventors: Robert H. Allen, [Sally] P. Stabler, and John Lindenbaum. (at page 7 of online text)

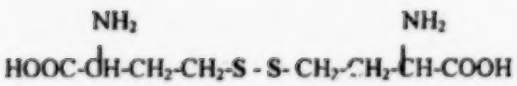
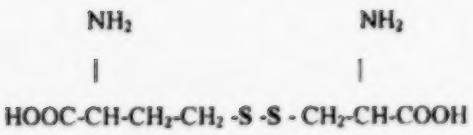
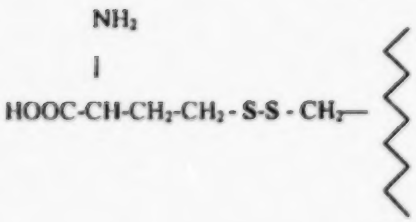
**Plaintiffs' Exhibit 33**

## Sulfhydryl Amino Acids in Human Serum

| Compound | Total Concentration (μM) | Percent of total (%) |
|---|---|---|
|  | | |
| **HOMOCYSTEINE** | 8 | 0.6 |
|  | | |
| **CYSTEINE** | 400 | 28.4 |
|  | | |
| **ALBUMIN** | 1000 | 71.0 |
| | 1408 | 100.0 |

**Plaintiffs' Exhibit 34**

# Forms of Homocysteine in Human Serum

| Compound | Percent of total (%) | Total Concentration (μM) |
|---|---|---|
|  | | |
| HOMOCYSTINE | 0.6 | 0.05 |
|  | | |
| HOMOCYSTEINE-CYSTEINE MIXED DISULFIDE | 28.4 | 2.27 |
|  | | |
| HOMOCYSTEINE-ALBUMIN | 71.0 | 5.68 |
| | 100.0 | 8.00 |

**Plaintiffs' Exhibit 35**

# Serum Valine and Methionine Levels in Pernicious Anaemia under Treatment

*J. Haemat., 1969, [16, 221]*

T.E. Parry

*Department of Clinical Pathology, Llaudough Hospital, United Cardiff Hospitals, Wales*

SUMMARY. The serum levels of the amino acids, valine and methionine have been determined by a quantitative microbiological method in 17 cases of pernicious anaemia both before and on successive days after treatment. The results have been compared with the serum levels of the two amino acids in 16 normal subjects under similar conditions.

The serum valine level is normal in pernicious anaemia in relapse. It falls sharply during the first 48 hours following treatment, thereafter rising gradually to reach about two-thirds the pretreatment level by the fifth day. This confirms the fall in valine level in whole blood and marrow previously reported in pernicious anaemia under treatment.

The serum methionine level is subnormal in pernicious anaemia in relapse. It rises to normal in 48 hours after treatment.

Both vitamin $B_{12}$ and folic acid are effective in lowering the serum valine and elevating the serum methionine.

The rise in serum methionine can be explained in terms of the known actions of vitamin $B_{12}$ and folic acid. The fall in serum valine cannot at present be explained but several possibilities are discussed.

* * * *

**Plaintiffs' Exhibit 41**

# Elevation of Total Homocysteine in the Serum of Patients with Cobalamin or Folate Deficiency Detected by Capillary Gas Chromatography-Mass Spectrometry

*J. Clin. Invest., Vol. 81, Feb. 1988, [466-474]*

Sally P. Stabler,* Paul D. Marcell,* Elaine R. Podell,* Robert H. Allen,* David G. Savage,** and John Lindenbaum**

**Divisions of Hematology and Oncology, Department of Medicine, and the Department of Biochemistry, Biophysics, and Genetics, University of Colorado Health Sciences Center, Denver, Colorado 80262; **and Department of Medicine, Harlem Hospital Center, Columbia-Presbyterian Medical Center and College of Physicians & Surgeons, Columbia University, New York, New York 10032*

**Abstract**

To determine if levels of serum total homocysteine are elevated in patients with either cobalamin or folate deficiency, we utilized a new capillary gas chromatographic-mass spectrometric technique to measure total homocysteine in the serum of 78 patients with clinically confirmed cobalamin deficiency and 19 patients with clinically confirmed folate deficiency. Values ranged from 11 to 476 μmol/liter in the cobalamin-deficient patients and 77 of the 78 patients had values above the normal range of 7-22 μmol/liter as determined for 50 normal blood donors. In the cobalamin-deficient patients, serum total homocysteine was positively correlated with serum folate, mean corpuscular volume, serum lactate dehydrogenase, serum methylmalonic acid, and the degree of neurologic involvement, and inversely correlated with platelets and hematocrit. In the folate-deficient

patients, values for serum total homocysteine ranged from 17 to 185 μmol/liter and 18 of the 19 patients had values above the normal range. Some patients with pernicious anemia who were intermittently treated with cyanocobalamin were found to have elevated serum levels of total homocysteine while they were free of hematologic and neurologic abnormalities. The measurement of serum total homocysteine will help define the incidence of cobalamin deficiency and folate deficiency in various patient populations.

* * * *

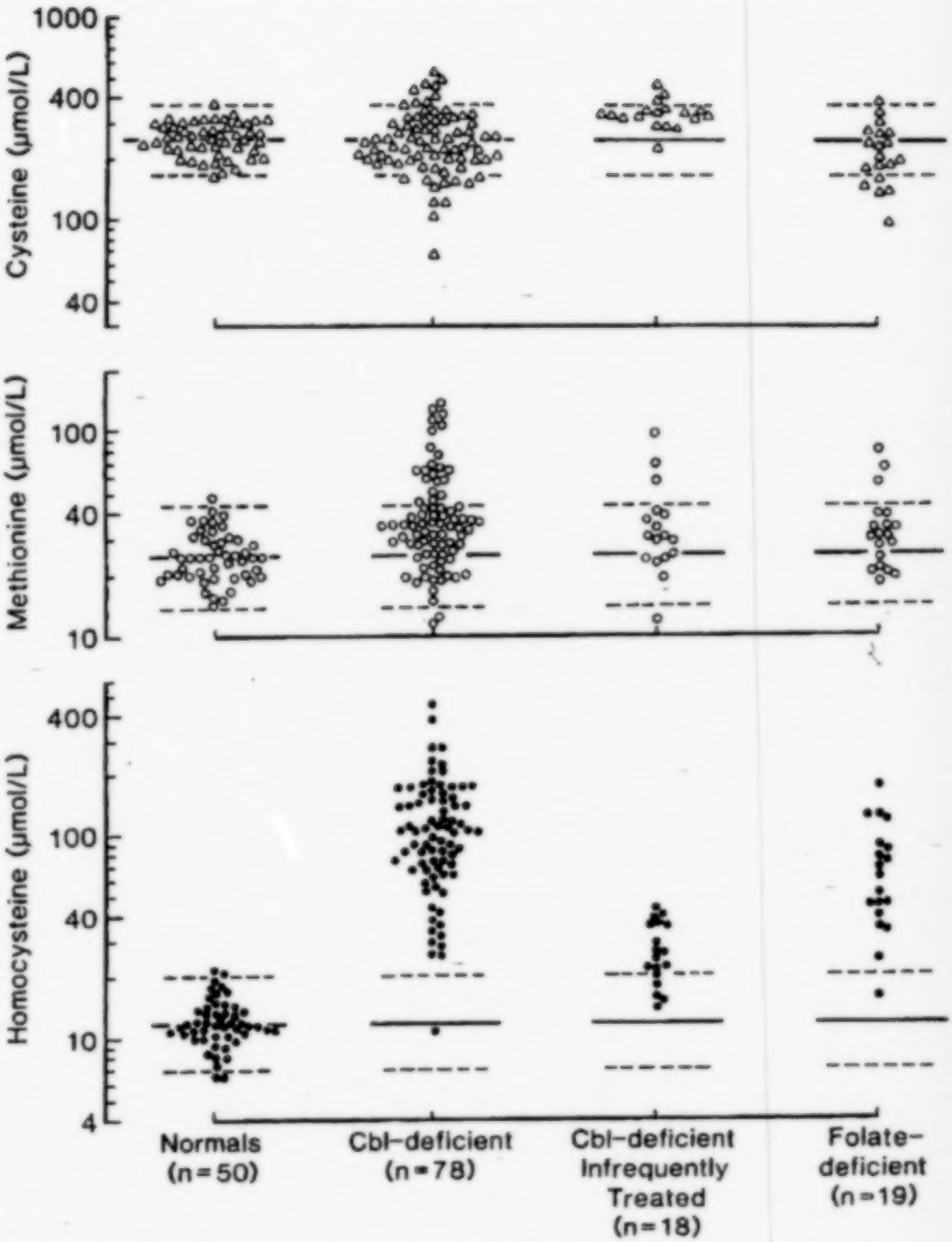

Cysteine (µmol/L)
1000
400
100
40
Methionine (µmol/L)
100
40
10
Homocysteine (µmol/L)
400
100
40
10
4
Normals (n=50)
Cbl-deficient (n=78)
Cbl-deficient Infrequently Treated (n=18)
Folate-deficient (n=19)

*Figure 2.* Levels of serum total homocysteine (*bottom*), serum methionine (*middle*), and serum total cysteine (*top*) in patients with clinically confirmed Cbl deficiency, folate deficiency, and Cbl-deficient infrequently treated patients who had no hematologic or neurologic abnormalities, using capillary gas chromatography-mass spectrometry. The normal range for total homocysteine is (7-22 μmol/liter), for methionine (14-44 μmol/liter), and for cysteine (173-378 μmol/liter). These ranges were calculated as the mean ± 2 SD after log transformation to correct for skewness towards higher values.

* * * *

**Plaintiffs' Exhibit 66**

# Cost-effectiveness of Vitamin Therapy to Lower Plasma Homocysteine Levels for the Prevention of Coronary Heart Disease[:] Effect of Grain Fortification and Beyond

*JAMA, August 22/29, 2001—Vol. 286, No. 8, [936-943]*

Jeffrey A. Tice, MD; Elizabeth Ross, MD; Pamela G. Coxson, PhD; Irwin Rosenberg, MD; Milton C. Weinstein, PhD; M.G. Myriam Hunink, MD, PhD; Paula A. Goldman, MPH; Lawrence Williams, MS; Lee Goldman, MD, MPH

**Context** A high homocysteine level has been identified as an independent modifiable risk factor for coronary heart disease (CHD) events and death. Since January 1998, the US Food and Drug Administration has required that all enriched grain products contain 140 μg of folic acid per 100 g, a level considered to decrease homocysteine levels.

**Objectives** To examine the potential effect of grain fortification with folic acid on CHD events and to estimate the cost-effectiveness of additional vitamin supplementation (folic acid and cyanocobalamin) for CHD prevention.

**Design and Setting** Cost-effectiveness analysis using the Coronary Heart Disease Policy Model, a validated, state-transition model of CHD events in adults aged 35 through 84 years. Data from the third National Health and Nutrition Examination Survey (NHANES III) were used to estimate age- and sex-specific differences in homocysteine levels.

**Intervention** Hypothetical comparison between a diet that includes enriched grain products projected to increase folic acid intake by 100 μg/d with the same diet without folic acid fortification; and a comparison between vitamin therapy that consists of 1 mg of folic acid and 0.5 mg of cyanocobalamin and the diet that includes grains fortified with folic acid.

**Main Outcome Measures** Incidence of myocardial infarction and death from CHD, quality-adjusted life-years (QALYs) saved, and medical costs.

**Results** Grain fortification with folic acid was predicted to decrease CHD events by 8% in women and 13% in men, with comparable reductions in CHD mortality. The model projected that, compared with grain fortification alone, treating all patients with known CHD with folic acid and cyanocobalamin over a 10-year period would result in 310000 fewer deaths and lower costs. Over the same 10-year period, providing vitamin supplementation in addition to grain fortification to all men aged 45 years or older without known CHD was projected to save more than 300000 QALYs, to save more than US $2 billion, and to be the preferred strategy. For women without CHD, the preferred vitamin supplementation strategy would be to treat all women older than 55 years, a strategy projected to save more than 140000 QALYs over 10 years.

**Conclusions** Folic acid and cyanocobalamin supplementation may be cost-effective among many population subgroups and could have a major epidemiologic benefit for primary and secondary prevention of CHD if ongoing clinical trials confirm that homocysteine-lowering therapy decreases CHD event rates.

* * * *

**Plaintiffs' Exhibit 87**

## Harrison (1987)
## Principles of Internal Medicine

"The finding of significant macrocytosis . . . suggests the presence of megaloblastic anemia. If the macrocytosis is marked (MCV >110 fl), the patient is much more likely to have a megaloblastic anemia."

"It should be emphasized that occasionally neurologic disease may occur in a patient with a normal hematocrit."

"The normal range of vitamin $B_{12}$ in serum is 200 to 900 pg/ml; values less than 100 pg/ml indicate clinically significant deficiency."

**Plaintiffs' Exhibit 89**

## Rakel (1984)
## Practice of Family Medicine

"The diagnosis of pernicious anemia should be suspected in an elderly individual who develops macrocytic, megaloblastic anemia while consuming a normal diet."

"Oval macrocytes are the characteristic abnormality in the blood and their presence results in a greatly increased value for the MCV."

"The anemia varies in degree but the blood hemoglobin level is less than 7 or 8 gm/dl in symptomatic patients."

"In vitamin $B_{12}$ deficiency, serum vitamin $B_{12}$ is greatly reduced (less than 100 pg/ml) whereas the serum folate is normal."

**Plaintiffs' Exhibit 91**

## Jandl (1987)
## Blood: Textbook of Hematology

". . . [the MCV is] usually over 115 fl but commonly 130-150 fl . . ."

". . . the white count . . . ordinarily is between 3,000 and 4,000 cells/ul . . ."

"Platelet counts are subnormal in nearly all patients . . ."

"LDH levels average about 15 times normal in megaloblastic anemia . . ."

"Concentrations [of serum $B_{12}$] below 100 pg/ml indicate vitamin $B_{12}$ deficiency sufficient to cause megaloblastic anemia."

**Plaintiffs' Exhibit 93**

## Cecil (1985)
## Textbook of Medicine

"Neurologic .symptoms occur later in some but not all patients. Curiously, they can occur in the absence of megaloblastic anemia."

"A decreased serum vitamin $B_{12}$ level is decisive diagnostic evidence. Clinical signs generally appear when the serum level is below 80 to 100 pg per milliliter."

"Methylmalonic aciduria is also a sensitive index of vitamin $B_{12}$ deficiency."

"In practice, assay of urinary methylmalonate is rarely necessary."

**Plaintiffs' Exhibit 95**

# Are Hematology and Medical Textbooks Correct in What They Tell us About the Clinical Spectrum of Cbl Deficiency?

**Approach**

1. Develop new methods for measuring metabolic parameters in Cbl deficient patients.

AND

2. Perform careful clinical studies with Cbl deficient patients.

**Plaintiffs' Exhibit 108**

# NEUROPSYCHIATRIC DISORDERS CAUSED BY COBALAMIN DEFICIENCY IN THE ABSENCE OF ANEMIA OR MACROCYTOSIS

*New England Journal of Medicine 318:1720-1728 (June 30), 1988*

* * * *

John Lindenbaum, M.D., Edward B. Healton, M.D., David G. Savage, M.D., John C.M. Brust, M.D., Thomas J. Garrett, M.D., Elaine R. Podell, B.A., Paul D. Marcell, B.S., Sally P. Stabler, M.D., and Robert H. Allen, M.D.

**Abstract** Among 141 consecutive patients with neuropsychiatric abnormalities due to cobalamin deficiency, we found that 40 (28 percent) had no anemia or macrocytosis. The hematocrit was normal in 34, the mean cell volume was normal in 25, and both tests were normal in 19. Characteristic features in such patients included paresthesia, sensory loss, ataxia, dementia, and psychiatric disorders; longstanding neurologic symptoms without anemia; normal white-cell and platelet counts and serum bilirubin and lactate dehydrogenase levels; and markedly elevated serum concentrations of methylmalonic acid and total homocysteine. Serum cobalamin levels were above 150 pmol per liter (200 pg per millileter) in 2 patients, between 75 and 150 pmol per liter (100 and 200 pg per milliliter) in 16, and below 75 pmol per liter (100 pg per milliliter) in only 22.

Except for one p[illegible]ho died during the first week of treatment, every patient in this group benefited from cobalamin therapy. Responses included improvement in neuropsychiatric abnormalities (39 of 39), improvement (often within

the normal range) in one or more hematologic findings (36 of 39), and a decrease of more than 50 percent in levels of serum methylmalonic acid, total homocysteine, or both (31 of 31).

We conclude that neuropsychiatric disorders due to cobalamin deficiency occur commonly in the absence of anemia or an elevated mean cell volume and that measurements of serum methylmalonic acid and total homocysteine both before and after treatment are useful in the diagnosis of these patients.

* * * *

Table 3. Neuropsychiatric Abnormalities and Responses to Treatment in 40 Patients.

| Abnormality | No. of Patients | Response to Cobalamin Therapy | | | |
|---|---|---|---|---|---|
| | | Complete | Partial | None | Unknown |
| Impaired vibration sense | 31 | 9 | 13 | 2 | 7 |
| Paresthesia | 30 | 21 | 9 | - | - |
| Impaired position sense | 22 | 11 | 7 | - | - |
| Impaired touch or pain perception | 17 | 9 | 6 | 1 | 1 |
| Ataxia | 17 | 12 | 4 | - | 1 |
| Abnormal gait | 15 | 7 | 5 | - | 3 |
| Memory loss | 13 | 8 | 3 | - | 2 |
| Decreased reflexes | 9 | 4 | 2 | - | 3 |
| Weakness | 9 | 8 | 1 | - | - |
| Decreased muscle strength | 7 | 5 | 2 | - | - |
| Psychiatric disorders | 7 | 5 | - | 1 | 1 |
| Romberg's sign | 7 | 3 | 3 | - | 1 |
| Fatigue | 6 | 6 | - | - | - |
| Urinary or fecal incontinence | 4 | 3 | 1 | - | - |
| Increased reflexes | 3 | 1 | 1 | - | 1 |
| Babinski's signs | 3 | - | - | 3 | - |
| Lhermitte's sign | 3 | 3 | - | - | - |
| Disorientation | 3 | 1 | 1 | - | 1 |
| Spasticity | 2 | - | 1 | 1 | - |
| Obtundation | 1 | - | - | - | 1 |
| Urinary urgency or nocturia | 1 | 1 | - | - | - |
| Impotence | 1 | 1 | - | - | - |
| Decreased vision or optic atrophy | 1 | 1 | - | - | - |

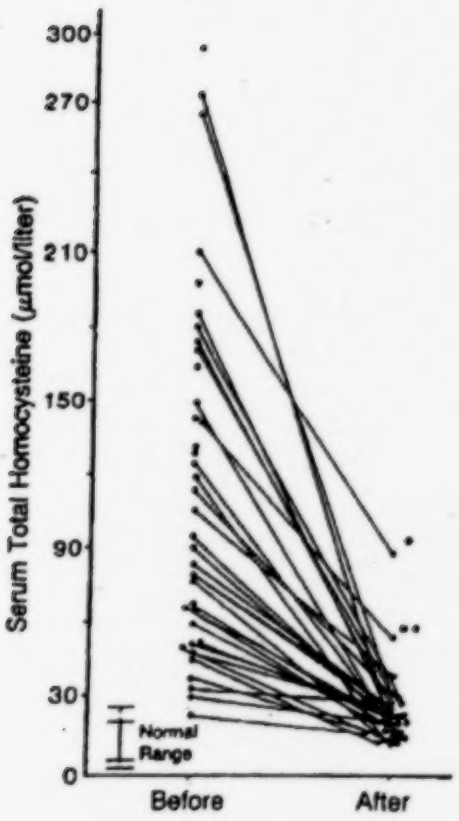



Figure 2. Serum Total Homocysteine Concentrations before and after Therapy with Cyanocobalamin.

The bars connected by unbroken lines indicates the normal range, on the basis of the mean ±2 SD (7 to 22 μmol per liter); the bars connected by broken lines indicate 3 SD above and below the mean (6 to 29 μmol per liter). One asterisk indicates serum obtained one day after a single injection of cyanocobalamin; two asterisks, serum obtained five days after a single injection.

* * * *

**Plaintiffs' Exhibit 109**

# EDITORIAL

## COBALAMIN AND THE NERVOUS SYSTEM

*New England Journal of Medicine 318:1752-1754 (June 30), 1988*

* * * *

The second report[6] considers problems that are anything but rare. In a large series of patients with "neuropsychiatric abnormalities," the authors conclude that such commonplace manifestations as paresthesias, ataxias, memory loss, weakness, personality and mood changes, and fatigue were due to cobalamin deficiency. The disorder was diagnosed according to laboratory tests, including assays for serum methylmalonic acid and homocysteine, the sensitivity of which, the authors suggest, made them notably helpful, since anemia was not present, the results of the Schilling test were normal, and serum cobalamin levels were not depressed. It is interesting and perhaps noteworthy that many of the patients were free of these traditional signs of cobalamin deficiency. If confirmed, this finding is important for obvious reasons. For one, it should influence the way we care for our patients. For another, it extends the perimeter of cobalamin-deficiency neuropathy to include much of what may be termed the neuropsychopathology of everyday life.

Here, perhaps, is the possible basis for a reservation or two. Symptoms such as those mentioned above are notoriously difficult to deal with quantitatively or to control in clinical experiments. They may have many causes, and as the authors acknowledge, they wax and wane. It also gives one pause to note that many of the patients supposed to have cobalamin deficiency had no recognizable cause of the

deficiency—and one need not be an obdurate skeptic to notice the authors' reliance on new diagnostic tests that gave abnormal results (many in patients with no known disease) when the results of more traditional tests were normal. The importance of such results will be weighed more easily as we gain experience with these procedures, which raise anew an old problem: How do we best diagnose a deficiency of a nutrient? It would appear that measurement of serum levels of the nutrient may not always be the answer, as many of us have suspected.

Finally, we are urged to look for cobalamin deficiency in patients with "unexplained" neuropsychiatric disorders (have any neuropsychiatric disorders been explained?), and if that is not possible, to administer cobalamin therapy anyway. This, of course, would add new costs to medical care, but if real benefits await these patients, the costs are justified. At the least, one can hope that physicians will try to gather meaningful data before giving everyone cobalamin in an uncritical manner, and that they will observe the results of cobalamin therapy in their patients. Although these findings are provocative and encouraging, we do need confirmatory data. Could it be that the many cobalamin injections over the years for vague symptoms were in fact justified?

Massachusetts General Hospital
Boston, MA 02114 WILLIAM S. BECK, M.D.

* * * *

## REFERENCES

* * * *

6. Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med 1988; 318:1720-8.

**Plaintiffs' Exhibit 112**

## Prospective Evaluation of the Clinical Spectrum of Cbl Deficiency

1. From 9/83 to 8/85 there were 7747 serum samples submitted for assay of Cbl at Harlem Hospital and Columbia Presbyterian Hospital in New York.

2. The serum Cbl was <200 pg/ml in 300 samples.

3. In the low Cbl group, 86 patients were judged to be Cbl deficient based on one or more objective responses to Cbl therapy.

**Plaintiffs' Exhibit 113**

## Responses to Cbl Therapy in 86 Consecutive Patients with Clinically Confirmed Cbl Deficiency

| Response | Percent |
|---|---|
| Increase in hematocrit >0.05 | 50 |
| Decrease in MCV >5 fl | 81 |
| Correction of peripheral smear | 48 |
| Clear neuropsychiatric improvement | 28 |
| 1 response | 33 |
| 2 responses | 31 |
| 3 responses | 33 |
| 4 responses | 3 |

(24/26 patients with neuropsychiatric responses also had one or more hematologic responses)

(85/86 patients had elevations of methylmalonic acid and homocysteine that corrected with Cbl therapy)

**Plaintiffs' Exhibit 114**

## Pretreatment Laboratory Tests in 86 Consecutive Patients with Clinically Confirmed Cbl Deficiency

| Test Result | Percent |
|---|---|
| Not Anemic | 44 |
| Not Macrocytic | 36 |
| Normal WBC | 84 |
| Normal Platelets | 79 |
| Normal Peripheral Smear | 32 |
| Normal LDH | 43 |
| Normal Bilirubin | 83 |
| Normal Gastrin | 37 |
| Absent IF Antibodies | 51 |
| Normal Schilling Test | 14 |
| Serum Cbl >100 pg/ml | 38 |

**Plaintiffs' Exhibit 122**

## Conclusions Concerning the Clinical Spectrum and Diagnosis of Cbl Deficiency

1. Much more varied than previously believed.
2. Neuropsychiatric abnormalities occur frequently in the absence of the classic hematologic abnormalities.
3. Serum Cbl levels are often >100 pg/ml and may be normal, even in patients with severe Cbl deficiency.
4. Serum methylmalonic acid and homocysteine levels are useful supplements to the serum Cbl assay.
5. Don't believe everything you read in medicine and subspecialty textbooks.
6. Even old fashioned diseases need to be restudied from time to time.

**Plaintiffs' Exhibit 125**

# Neuropsychiatric Consequences of Cobalamin Deficiency

*Advances in Internal Medicine (Stollerman, ed.) Vol. 36, [33-56]*

*1991*

William S. Beck, M.D.

*Professor of Medicine, Department of Medicine, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts*

## Editor's Introduction

The history of vitamin $B_{12}$ is a 20th-century classic in clinical investigation: inquiry at the bedside, self-experimentation, integration of vexing observations on domestic and laboratory animals and microbiologic species, all culminating in sophisticated chemical identification and synthesis of the cobalamin molecule. Pernicious anemia and other well-known forms of vitamin $B_{12}$ deficiency were promptly and unequivocally responsive to the pure vitamin.

Included were some of the neuropsychiatric changes provided they were of reasonably short duration. The more chronic neuropsychiatric syndromes did not show dramatic responses. Claims to the contrary were based on anecdotal reports and uncontrolled studies that such commonplace complaints as memory loss, weakness, fatigue, and personality and mood changes were benefited.

The question has been reopened, however, by new studies, which appear valid, in which improvement has been described in neuropsychiatric syndromes by the use of vitamin $B_{12}$ even in patients in whom there is no anemia and in whom some of the conventional assays for vitamin $B_{12}$

deficiency have yielded either equivocal or normal results. Now, with improved methods and new insights into possible advantages of different forms of cobalamin, there is renewed ferment after 30 years of, it appears, unwarranted complacency.

For the past 35 years, Dr. Beck has been in the forefront of studies on Vitamin $B_{12}$, its chemistry and physiology, and is without equal as an objective authority in providing a critical and valuable appraisal of what may be expected from these provocative recent studies.

*Gene H. Stollerman, M.D.*

* * * *

[Excerpt from Dr. Beck's chapter:]

**Serum Methylmalonic Acid and Homocysteine.**

* * * *

More recent work[80] indicates that 95% of patients who relapse because of suboptimal therapy display early elevations of serum methylmalonic acid, total homocysteine, or both metabolites, compared with 69% with low serum cobalamin levels. Of 419 consecutive patients with recognized significant cobalamin deficiency, 12 had serum cobalamin levels greater than 200 pg/mL, mild or absent anemia, and (in 5) prominent neurologic signs that responded to cobalamin. In all 12 cases, both serum methylmalonic acid and total homocysteine values were increased. Observations by others[81] confirm the value of total homocysteine assays in cobalamin deficiency. Such data, it would appear, establish that serum cobalamin is normal in a significant minority of cobalamin-deficient patients and that assay of these serum metabolites is diagnostically essential.

* * * *

**Commentary**

For years, we have seen inconclusive reports suggesting that neurologic symptoms attributable to cobalamin deficiency are more varied and complex than those of subacute combined system disease. Needless to say, the failure of early observers to prove a causal connection between cobalamin deficiency and certain neurologic symptoms did not prove there is no connection.

Such proof may be at hand. The new work summarized above strongly supports the claim that determinations of serum methylmalonic acid and total homocysteine provide a new diagnostic standard against which other procedures are henceforth to be compared and evaluated. The use of these tests has provided compelling evidence that intracellular cobalamin deficiency can and often does exist in the absence of megaloblastic anemia or low serum cobalamin levels.

* * * *

**Plaintiffs' Exhibit 126**

## 37 Consecutive Alcoholic Patients with Anemia and Megaloblastic Bone Marrows

| | | |
|---|---|---|
| Folate | < 2.1 ng/ml | 15/37 (41%) |
| Homocysteine | > 22 μM | 34/37 (92%) |
| | > 30 μM | 27/37 (73%) |

**Plaintiffs' Exhibit 132**

**CONFIDENTIAL**

LICENSE AGREEMENT

THIS AGREEMENT, made effective as of this 22nd day of November 1988, by and between UNIVERSITY PATENTS, INC., a corporation of the State of Delaware (hereinafter referred to as "UPI"), and METABOLITE LABORATORIES, INC., a Colorado corporation, having a place of business at No. 4001 S. Dexter Street, Inglewood, Colorado 80110 (hereinafter referred to as "LICENSEE");

WITNESSETH:

WHEREAS, ROBERT H. ALLEN and SALLY P. STABLER, members of the faculty and staff of the UNIVERSITY OF COLORADO, and JOHN LINDENBAUM, a member of the faculty and staff of COLUMBIA UNIVERSITY, are co-inventors of United States Patent Application Serial No. 933,553, filed on November 20, 1986 and entitled: "Assay For Sulfhydryl Amino Acids and Methods for Detecting and Distinguishing Cobalamin and Folic Acid Deficiency", a copy of which is appended hereto and marked "Attachment 1"; and

WHEREAS, the portion of the Invention developed by ROBERT H. ALLEN and SALLY P. STABLER is the property of UNIVERSITY OF COLORADO FOUNDATION, INC., and the portion of the Invention developed by JOHN LINDENBAUM is the property of THE TRUSTEES of COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK: and

WHEREAS, UPI has the exclusive right to license to others the aforementioned United States Patent Application, together with any patents which may issue thereon throughout the world, as evidenced by a certain Service Agreement by and between UNIVERSITY OF COLORADO FOUNDATION, and THE TRUSTEES OF COLUMBIA UNI-

VERSITY IN THE CITY OF NEW YORK and UPI, a copy of which is appended hereto and marked "Attachment 2"; and

WHEREAS, LICENSEE intends to conduct the business of a reference laboratory whereunder it will perform diagnostic assays for physicians and for commercial diagnostic laboratories, reporting to said physicians and laboratories results of said assays for use by said physicians and transmittal by said laboratories to its customers; and

WHEREAS, LICENSEE desires to obtain a nonexclusive license from UPI under such patent application and patents;

NOW THEREFORE, in consideration of these premises and of the mutual covenants hereinafter set forth, UPI and LICENSEE mutually covenant and agree as follows:

## ARTICLE I - DEFINITIONS

For the purpose of this Agreement, the following terms shall have the indicated meanings:

1.01 The term "Invention" shall mean any and all inventions made by ROBERT H. ALLEN and SALLY P. STABLER at the UNIVERSITY OF COLORADO and JOHN LINDENBAUM at COLUMBIA UNIVERSITY disclosed in a certain United States patent application entitled: "Assay for Sulfhydryl Amino Acids and Methods for Detecting and Distinguishing Cobalamin and Folic Acid Deficiency".

1.02 The term "Licensed Patents" shall mean United States Patent Application 933,553 filed on November 20, 1986 and entitled: "Assay for Sulfhydryl Amino Acids and Methods for Detecting and Distinguishing Cobalamin and Folic Acid Deficiency", including any patents issuing therefrom, and continuations, continuations-in-part, divisions and/or reissues thereof and to which UPI has or shall have the right to grant licenses during the term hereof.

1.03 The term "Licensed Process(es)" shall mean any assays conducted in accordance with the Invention.

1.04 The, term "Sales" shall mean the amount charged by a physician or commercial diagnostic laboratory to his patient or its customer for providing to said patient or customer the results of any assay performed by LICENSEE in accordance with the Invention, without any deduction therefrom.

1.05 "Confidential Information" shall mean all information, expertise, and technology relating to the formulation, development and commercialization of Licensed Process which is communicated to LICENSEE hereunder (including communications by or from ROBERT H. ALLEN to LICENSEE pursuant to any agreement between them or between LICENSEE and the UNIVERSITY OF COLORADO), except for such part thereof that was, at the time of the disclosure, already known to LICENSEE, patented, previously disclosed in a publication, or otherwise in the public domain, or such part thereof that subsequently becomes patented, disclosed to LICENSEE or in a publication by a third party having a right to make such disclosure or otherwise becomes within the public domain through no fault of the receiving party.

1.06 The term "License Year" shall mean a twelve month period of time commencing on the first day of January, April, July or October next following the date of this Agreement, and each twelve (12) month period thereafter.

## ARTICLE II - GRANT BY UPI

2.01 Subject to the terms and conditions of this Agreement and to the provisions of 35 USC 200-212 as implemented by 37 CFR part 401, a copy of which is appended hereto as "Attachment 3", UPI agrees to grant and hereby grants to LICENSEE a nonexclusive license under the Licensed Patents, which license shall be for the practice of Licensed Process in the United States.

2.02 The license hereby granted by UPI shall not include the right of LICENSEE to grant any sublicenses.

2.03 If UPI shall hereafter grant, at any time during the term of this Agreement, a license which grants rights

granted hereunder to LICENSEE under the Licensed Patents at a more favorable royalty rate or any other more favorable terms than those provided for in this Agreement, LICENSEE shall be entitled to receive the same more favorable royalty rate or any other more favorable terms specified with respect to such Licensed Patents, subject to the other less favorable terms and conditions under which such more favorable royalty rate or other terms have been granted, provided that any such modification of this Agreement, which shall be by written amendment, shall be effective only for the period such more favorable royalty rate or other more favorable terms are in effect under such license. The foregoing shall not apply:

(1) where UPI receives monetary consideration for such license; or

(2) where LICENSEE elects to continue paying royalties on the basis set forth in this Agreement.

ARTICLE III - REMITTANCES UNDER THE LICENSE

3.01 In consideration of the grant made in ARTICLE II, LICENSEE agrees to pay UPI in respect to Licensed Processes conducted by or for LICENSEE a royalty in an amount equal to six percent (6%) of the cumulative Sales of LICENSEE'S customers, excepting therefrom such customers who are licensees of UPI under the Licensed Patents and who have direct royalty obligations to UPI thereunder.

**Plaintiffs' Exhibit 143**

University of Colorado Health Sciences Center
Department of Medicine
Division of Hematology

Campus Box B170
4200 East Ninth Avenue
Denver, Colorado 80262
(303) 270-8474

School of Medicine

June 4, 1992

Dr. Peter W. Wentz
Director of Toxicology
Roche Biomedical Laboratories
1447 York Court
Burlington, NC 27296

Dear Peter,

As we discussed on the telephone yesterday, I am very pleased that things are running smoothly with you in terms of the performance of the metabolite assays. I will always be glad to answer any technical questions that arise concerning the metabolite assays and Dr. Sally P. Stabler and myself will be glad to answer any questions that physicians might have regarding the results or interpretations of the metabolite assays.

One aspect of the metabolite assays that I did not mention in our conversations concerns the occasional request that only one of the metabolites be performed rather than all four. I have always refused to do this because I do not believe that it is good medical practice. My reasoning is based on the fact that a normal value for one metabolite does not exclude the possibility that one or more of the additional metabolites may be elevated as well as the fact that an isolated elevated

value for one metabolite can not be interpreted completely without knowing whether the other metabolites are also elevated or are normal. There are also technical and possible economic reasons for not separating the metabolite assays but these are overshadowed by the importance of good medical practice as just noted above. If you have any questions about this aspect of the metabolites or if this becomes an issue in the future, I hope you will feel free to ask me for any assistance that I might provide in this matter.

I am also enclosing four tables that might serve as a starting point for a brochure or physician education piece about the indications, utility and interpretation of the metabolite assays. You may want to alter the normal ranges that I have listed for serum vitamin $B_{12}$ and folate to match those of Roche Biomedical Laboratories and I hope that you and your marketing people will feel free to make any other changes that you believe would be helpful. I am also enclosing reprints of several articles that you may wish to list as a bibliography at the end of an education piece regarding the metabolite assays.

Please let me know if I can be of any further assistance.

With best regards,

Sincerely yours,

/s/

Robert H. Allen, M.D.
Professor of Medicine
Professor of Biochemistry
Director, Division of Hematology

RHA:vla
Enclosures

**Plaintiffs' Exhibit 144**

# FAX COVER SHEET

University of Colorado Health Sciences Center

DATE: August 25, 1992

TO: Dr. Peter W. Wentz, Director of Toxicology

LOCATION: Roche Biomedical Laboratories

PHONE NUMBER: 1-800-334-5161

FAX NUMBER: 1-919-584-0865

FROM: Dr. Robert H. Allen

FAX: (303) 270-8477

NUMBER OF PAGES INCLUDING COVER SHEET: 7

MESSAGE: Dr. Allen would like for you to call him once you have had a chance to review the attached articles. Thank You!

Sent by: Valarie Allen
Secretary
Hematology Division

IF YOU DO NOT RECEIVE ALL OF THE PAGES, PLEASE CALL THE DIVISION OF HEMATOLOGY AS SOON AS POSSIBLE AT (303) 270-8474. THANK YOU.

*The Wall Street Journal*
Wednesday, August 19, 1992

**TECHNOLOGY & HEALTH**

# *Certain Amino Acid Increases the Risk Of Heart Attacks*

* * * *

## New Data Say Most Vulnerable Are People With Poor Diets And Genetic Predisposition

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By David Stepp

*Staff Reporter of the Wall Street Journal*

BOSTON – A study here showed that high levels of a rarely measured blood constituent are associated with a tripling of heart-attack risk.

The finding suggests that the substance, homocysteine, poses substantial risk of heart disease in people with poor diets or with a genetic predisposition to high levels of the chemical.

But the risk can be offset by dietary supplements of vitamin B-6 or a related vitamin called folic acid, which help metabolize homocysteine, said Mier Stampfer, a researcher at Brigham and Women's Hospital here and co-author of the report. The report appears in today's issue of the Journal of the American Medical Association.

### Heart Disease at Early Age

Homocysteine, an amino acid, is normally formed in the body as proteins are broken down. In previous studies, scientists found that its levels are elevated in people with an inherited disease, called homocysteinuria, which causes heart disease at an early age, said Dr. Stampfer. High levels of

homocysteine also have been previously linked to increased risk of stroke. Scientists believe that the substance is toxic to cells lining the walls of blood vessels.

In the latest study, researchers analyzed blood samples from 14,916 male physicians that were collected as part of a larger research project called the Physicians Health Study. In a five-year follow-up, 271 of the doctors had heart attacks. A comparison of homocysteine levels in the men who suffered heart attacks, with levels in those who didn't, showed that men with markedly elevated amounts of the substance were 3.4 times more likely to have attacks.

Only about 5% of the men were in the high-risk category. Their high levels of homocysteine probably represented "a mixed bag" of metabolic problems and dietary deficiencies, rather than cases of homocysteinuria, said Dr. Stampfer.

**'Well-Fed Doctors'**

"These were well-fed doctors," he added. "In the general population, maybe 10% have higher-than-optimal levels of homocysteine," due to a greater proportion of vitamin deficiency than experienced among the doctors. Vitamin B-6, the corrective for high homocysteine levels, is found in cereal grains, vegetables, milk, yeast and other foods.

Dr. Stampfer said that high levels of homocysteine may account for at least some of the cases of early heart attacks that aren't associated with major risk factors, such as smoking or high cholesterol levels. He added that unlike the pattern of risk for other contributors to heart disease, the risk associated with homocysteine doesn't appear to rise gradually as levels of the substance go up. Instead, the elevated risk was present in men with abnormal homocysteine levels above a certain threshold.

More research is needed to establish whether heart-attack risks in people with high homocysteine can be lowered by vitamin supplements, Dr. Stampfer cautioned. However,

even if that is proven, making practical use of the finding may be difficult; currently, few hospitals or medical laboratories are equipped to measure homocysteine levels.

* * * *

## A Prospective Study of Plasma Homocyst(e)ine and Risk of Myocardial Infarction in US Physicians

*JAMA, August 19, 1992— Vol 268, No. 7 at 877-881*

Meir J. Stampfer, MD; M. Rene Malinow, MD; Walter C. Willett, MD; Laura M. Newcomer; Barbara Upson; Daniel Ullmann, MPH; Peter V. Tishler, MD, Charles H. Hennkens, MD

**Objective.**-To assess prospectively the risk of coronary heart disease associated with elevated plasma levels of homocyst(e)ine.

**Design.**-Nested case-control study using prospectively collected blood samples.

**Setting.**-Participants in the Physicians' Health Study.

**Participants.**-A total of 14916 male physicians, aged 40 to 84 years, with no prior myocardial infarction (MI) or stroke provided plasma samples at baseline and were followed up for 5 years. Samples from 271 men who subsequently developed MI were analyzed for homocyst(e)ine levels together with paired controls, matched by age and smoking.

**Main Outcome Measure.**-Acute MI or death due to coronary disease.

**Results.**-Levels of homocyst(e)ine were higher in cases than in controls (11.1±4.0 [SD] vs 10.5±2.8 nmol/mL; $P$=.03). The difference was attributable to an excess of high values among men who later had MIs. The relative risk for the highest 5% vs the bottom 90% of homocyst(e)ine levels was 3.1 (95% confidence interval, 1.4 to 6.9; $P$=.005). After

additional adjustment for diabetes, hypertension, aspirin assignment, Quetelet's Index, and total/high-density lipoprotein cholesterol, this relative risk was 3.4 (95% confidence interval, 1.3 to 8.8) ($P$=.01). Thirteen controls and 31 cases (11%) had values above the 95th percentile of the controls.

**Conclusions.**-Moderately high levels of plasma homocyst(e)ine are associated with subsequent risk of MI independent of other coronary risk factors. Because high levels can often be easily treated with vitamin supplements, homocyst(e)ine may be an independent, modifiable risk factor.

* * * *

Elevated homocyst(e)ine levels can often be normalized by modest doses of folate (1 to 5 mg/d).[30,34] For cases that are resistant to this therapy, the addition of vitamin $B_6$, choline, or betaine is often effective.[35,36] These supplements have few or no side effects under most circumstances.

* * * *

**Plaintiffs' Exhibit 145**

**Roche Biomedical Laboratories**
a subsidiary of Hoffman-LaRoche Inc.

October 30, 1992

Roche Biomedical Laboratories, Inc.
P.O. Box 2230
Burlington, North Carolina 27216-2230

Robert H. Allen, M.D.
UC Health Sciences Center
Campus Box B170
4200 East Ninth Avenue
Denver, Colorado 80262

Telephone: 919-584-5171
Cable: Biomed

RE: Serum Homocysteine Ordered as a Single Test Comment to Accompany Report

Dear Bob,

As we discussed on the telephone recently, a separate market may develop for homocysteine as a marker for the development of premature CHD as the result of recent journal articles. The concern for good medical practice that you expressed regarding possible misinterpretation based on only partial metabolite data is well taken.

If we are to offer homocysteine as a single test, it may be advisable to have a canned comment appear on the patient report indicating test limitations and suggesting possible followup testing. With that in mind, the following comment is for your perusal.

> **NOTE: Moderately high levels of serum homocysteine are associated with subsequent risk of myocardial infarction independent of other coronary risk factors.[1] If, however, the test was ordered to evaluate**

**any unexplained hematologic abnormality, a normal or abnormal result is not conclusive, and it is recommended to order the complete Megaloblastic Anemia Profile #706960.**

**[1]Stampfer, MJ, et al, A Prospective Study of Plasma Homocysteine and Risk of Myocardial Infarction in US Physicians, JAMA, 1992; 268:877-881.**

I would appreciate your comments on this and any other suggestions for promoting this test.

Thanks for your assistance.

Sincerely,

/s/_____
Peter W. Wentz, PhD
Director of Toxicology

**Plaintiffs' Exhibit 159**

*Laboratory Corporation of America Holdings*
231 Maple Avenue
Burlington. North Carolina 27215
Telephone: 1-800-222-7566, ext. 4176
FAX: 910-222-1568

November 2, 1998

Dr. Robert H. Allen
Metabolite Laboratories, Inc.
301 Garfield St., Unit 2-West
Denver, CO 80206

Re: Royalty Agreement Dated January 31, 1991

Dear Dr. Allen:

Attached is the calculation for the 3rd quarter 1998 BDS payment. Test number 706994 (homocysteine) has been removed for August 1998 and September 1998. This is due to a change in methodology in performing this test. Also, the payment has been reduced for an overpayment for this same test number for August on the LCBS.

I hope the schedule is clear so you can see the earnings from 3rd quarter BDS and the recoupment of the August 1998 LCBS.

If you have any questions, please give me a call.

Sincerely,

/s/ Denise A. Hodge
Denise A. Hodge
Revenue Reporting

**Plaintiffs' Exhibit 165A**

TIME. 0000 VOL 0000
DOB:
00/00/00
FASTING:
000-000-0000

TESTING N 000/00

LABORATORY CORP
OF AMERICA

[PATIENT NAME]
[PATIENT ADDRESS]
[PATIENT ADDRESS]

05/19/99

HOMOCYST(E)INE, PLASMA/SERUM

| | | | |
|---|---|---|---|
| Homocyst(e)ine, P/S | – | 16.0 HI mcmol/L | 5.0 – 15.0 BN |

This test is intended to be used as an independent risk factor for vascular disease and is NOT intended for the diagnosis of folate or vitamin B12 deficiency. LabCorp has available the Megaloblastic Anemia Profile, Serum for that purpose.

Homocyst(e)ine Reference Ranges

| | |
|---|---|
| Normal: | 5 – 15 |
| Desirable: | <10 |

LAB: BRN LABCORP BURLINGTON DIRECTOR: Frank Hancock MD
1447 YORK COURT BURLINGTON NC 27218-2230
BRANCH PHONE: 800-762-4344 LAB PHONE: 336-584-5171

LAST PAGE OF REPORT

**Plaintiffs' Exhibit 178**

# THE HEART REVOLUTION

## The Extraordinary Discovery That Finally Laid the Cholesterol Myth to Rest and Put Good Food Back on the Table

Kilmer McCully, M.D.
and Martha McCully

HarperPerennial
*A Division of* HarperCollins *Publishers*

[1999]

* * * *

### The Heart of the Theory

In 1969 I first proposed the homocysteine theory of heart disease. When there is too much homocysteine in the blood, arteries are damaged and plaques form. The result is arteriosclerosis and heart disease. This happens when we don't get enough of certain vitamins—namely B6, B12, and folic acid. These B vitamins are missing in our diets because processing and refining foods (think white flour, sugar, and canning) destroys these sensitive vitamins.

When I first started my research, homocysteine was an obscure minor amino acid known only to biochemists for its function in protein metabolism. Before the disease homocystinuria was discovered to 1962, medical scientists had no clue that homocysteine could be a key player in the most important disease in the population—arteriosclerosis.

Of course, besides diet there are many other factors that can increase homocysteine in our blood: genetic background, certain drugs, aging, hormonal changes such as menopause, smoking, how little we exercise, diabetes, and

high blood pressure. We can't control all these things. But we can do something about our diet, and our diet is the one sure way to keep homocysteine levels low.

How do you eat? Is your diet mostly meat and potatoes? Is it low-fat and high-carbohydrate? Are you a vegetarian? A vegan? Do you follow a high-protein plan? How you eat affects how your body prevents disease.

Often it's the balance of what you eat that needs adjustment. For example, an amino acid called methionine is one of the essential building blocks of all proteins in foods. It is especially abundant in meats and dairy products. In the body, methionine is normally converted to homocysteine. We need some methionine, but an excessive amount will create too much homocysteine, damaging the arteries. The good news is that homocysteine can also be converted back to methionine or excreted from the body by the three

* * * *

**Proof of the Homocysteine Theory**

In the 1990s the results of some very important studies proving the homocysteine theory were discussed in the national media. Unlike the cholesterol hypothesis, the homocysteine theory has been proven by experimental studies with animals, studies of homocysteine levels in patients with heart disease and stroke, and studies of populations that are susceptible to arteriosclerosis. As discussed previously, the arteriosclerosis produced in monkeys fed a diet deficient in vitamin B6 was interpreted as the result of increased blood homocysteine levels. To test this interpretation, homocysteine was infected into rabbits, baboons, and other animals. Just as predicted, these experiments show that homocysteine causes plaques to form in arteries and blood clots within veins and arteries. Plaques are a thickening on the artery wall caused by an overgrowth of muscle cells and deposits of fibrous tissue. What role does homocysteine play? Homocysteine affects this process in several ways. It damages the cells lining the artery walls, stimulating over growth of muscle cells. Homocysteine also releases a substance that destroys the elastic tissue of the artery. The

final result is a thickened, tough, inelastic artery wall. With time, the plaques develop calcium deposits. The experiments also clearly show that the fats and cholesterol within plaques are a complication, not a cause, of arteriosclerosis. If butter or cholesterol is given to animals that are infected with homocysteine, the plaques in the arteries will contain deposits of fats and cholesterol. However, the fats and cholesterol are not *causing* the damage, they are only being deposited where damage has already occurred. If no fat or cholesterol is added to the diet, the plaques don't contain fat or cholesterol, but the plaques are destructive nonetheless since they narrow the arteries.

Of course, the most powerful proof is in human studies. Some 10-40 percent of patients with vascular disease in clinics and hospitals worldwide has consistently been shown to have high levels of homocysteine. Up to two-thirds of the elderly are deficient in either B6, B12, or folic acid, as shown by the Framingham Heart Study. Patients with deficiencies of folic acid have high levels of homocysteine in their blood all the time. Others with a deficiency of B6 will only have large increases in homocysteine in their blood within a few hours after meals. If a person is deficient in folic acid *and* vitamin B6, blood homocysteine will be elevated both before and after meals. Studies have shown that folic acid keeps blood homocysteine levels low by converting it back into methionine. Vitamin B6 turns homocysteine into other substances that are excreted from the body in the urine.

Here are some examples of the large-scale population studies definitively showing that elevation of blood homocysteine is associated with increased risk of heart attack and heart disease.

- In February 1998 investigators at the Harvard School of Public Health published the results of the Nurses' Health Study, one of the studies that originally set out to prove that cholesterol was causing heart disease. Instead this study has shown that deficiencies of B vitamins are doing the damage. During a fourteen-year period, 80,000 participants answered question-

naires about their food consumption. The study revealed that those nurses with the lowest consumption of folic acid and B6 had the highest death rates from cardiovascular disease and heart attack.

- In April 1998 investigators from England published a study of 21,500 men who were followed for almost nine years. Blood homocysteine levels were higher in men who died of heart disease than in men who did not. The higher the blood homocysteine level, the higher the risk of dying from heart disease.

- The Physicians Health Study, completed in 1992, showed that among the 14,000 participants, those with high homocysteine were three times more likely to have a heart attack during a five-year period than persons with normal levels.

- In the 1996 Nutrition Canada Study of 5,000 people studied for fourteen years, those with the lowest levels of folic acid in the blood were almost twice as likely to die from heart disease as those with the highest levels.

- A study from Norway showed that among 587 patients with proven coronary heart disease, risk of death is directly related to the level of homocysteine in the blood. Cholesterol level, on the other hand, did not predict the risk of death.

- The Hordaland Study of 16,000 residents of Bergen, Norway, showed that homocysteine increases in the presence of other known risk factors for heart disease, including male gender, old age, smoking, high blood pressure, elevated cholesterol level, and lack of exercise.

- A multicountry study in 1997 showed that the death rate from coronary heart disease is directly related to blood homocysteine levels. In northern European countries, where heart disease is frequent, people have higher blood levels of homocysteine than in southern

European countries, where heart disease is less frequent.

The study in Hordaland, Norway, is especially interesting because a variety of factors associated with heart disease were related to high blood homocysteine. Known risk factors for heart disease—aging, male gender, menopause, lack of vegetables and fruits or vitamin supplements, lack of exercise, high blood pressure, and smoking—cause homocysteine levels to rise. The studies from Canada and the Nurses Health Study show directly that death from heart disease is related to dietary deficiencies of folic acid and vitamin B6. The Physicians Health Study and the British study show that elevated blood homocysteine increases the risk of dying from heart disease. All these studies are powerful evidence supporting the validity of the homocysteine theory of heart disease,

There are, in fact, many cases of heart disease in which cholesterol levels are quite normal. Usually these patients have high homocysteine that is independent of cholesterol; remember J.E. from the first case study. In 1990 a study at the Providence V.A. Medical Center showed that the patients with the most advanced arteriosclerosis typically had normal cholesterol levels, and two-thirds of all these patients had no hypertension, diabetes, or elevated cholesterol at all. Only about 15 percent of cases with severe arteriosclerosis had high cholesterol. Eating a cholesterol-rich diet doesn't cause heart disease, and measuring blood levels of cholesterol fails to predict heart disease in most of those who have it.

Another significant factor can affect homocysteine levels. About 12 percent of the population worldwide carry a genetic defect affecting the ability to metabolize homocysteine normally. All of us have an enzyme, methylenetetrahydrofolate reductase, that combines with folic acid to lower homocysteine levels. People born with an abnormality of this enzyme need to consume even more folic acid than normal to keep homocysteine levels in check. If folic acid intake is deficient, people with this

genetic defect have a greater chance than average of developing arteriosclerosis and heart disease because of elevated homocysteine levels. If you have a strong family history of heart disease, it is worth checking to find out if you have this enzyme abnormality. Specialized biochemical and genetic tests available in commercial laboratories and medical centers can detect this abnormality. I'll talk about how much folic acid you should take in Chapter Five.

### An Action Plan: Prevention and Therapy of Heart Disease

Now we know what causes heart disease, and how homocysteine works. We even know what can be done to lower homocysteine levels, which you'll learn throughout this book. And it's not just for prevention. If you already have heart disease, vitamin therapy can lower homocysteine and stop the disease from progressing. A 1998 clinical study from Canada offers the first proof that B vitamin therapy can prevent arteriosclerosis from getting worse. Patients with elevated homocysteine and arteriosclerosis of arteries to the brain were given intensive therapy with folic acid, vitamin B6, and vitamin B12. The plaques stopped progressing and the homocysteine levels returned to normal. In a 1996 study of patients with coronary heart disease, therapy with vitamin B6, folic acid, B12, and other nutrients slowed the development of the disease.

The amount of these vitamins needed to keep homocysteine in check (3 milligrams of B6 and 400 micrograms of folic acid) is provided by the Heart Revolution diet. Luckily, homocysteine levels can be lowered simply and safely by using B vitamins. And it's never too late to start. We knew B vitamins were important before these studies, but now we understand *why* they are so vital.

In 1995 a group of patients being treated for carpal tunnel syndrome ended up reaping the benefits of the homocysteine approach. This common painful condition is caused by overuse of the wrist, hand, and fingers

(typists and tennis players are typical sufferers). In other cases the condition is related to pregnancy, diabetes, or hormonal disturbances. Patients were given large doses of B6 to alleviate the symptoms. Most of these patients were helped, but the study also revealed that these patients had a far lower incidence of heart disease than would have been expected. In addition, the study found that many of these patients were originally deficient in vitamin B6 in the blood, which for many had produced symptoms of heart disease as well as carpal tunnel syndrome.

All of these examples point to the key role B vitamins play in the health of the body. But the purpose here is to make sure you get enough of them in your diet to prevent heart disease. In the next several chapters you'll see how the American diet has failed to provide adequate nutrients. You'll also read about ways to improve your diet so that you can protect yourself.

The American public is ready for a revolutionary way of thinking. Once we change our ideas about what causes heart disease, we can improve our diets, prevent disease, and live the healthier life that is within our grasp.

**Plaintiffs' Exhibit 180**

## Homocyst(e)ine *The Bad Amino Acid?*

By Peter W. Wentz, PhD

[1999]

### Introduction

The pioneering work of Dr. Kilmer McCully has led to the development of the homocyst(e)ine theory of arteriosclerosis. The theory accounts for many of the causes of severe arteriosclerosis in patients with lifelong normal or desirable cholesterol levels (<200 mg/dL). It is estimated that an elevated blood homocyst(e)ine level may account for at least 10 percent of the risk of coronary heart disease in the U.S. population.

Cross-sectional, epidemiological and case-control studies have confirmed the relationship between elevated homocyst(e)ine levels and the risk of atherosclerotic disease. No causal relationship has been established, but studies are under way to determine whether lowering homocyst(e)ine will prevent occlusion in patients at risk for cardiovascular, cerebrovascular and peripheral vascular disease.

### Atherogenic Effect of Homocyst(e)ine

According to the homocyst(e)ine theory, the origin of arteriosclerosis is the toxic effect of homocyst(e)ine on the endothelial lining of arteries, which causes plaque formation. Evidence for this has come from in vitro studies in human cell cultures and from in vivo animal models. The mechanism of action is unclear but may be related to the formation of LDL- homocyst(e)ine aggregates, which are taken up by macrophages of the artery wall to form the foam cells of early arteriosclerotic plaques. For a recent review of the hypothesized mechanisms of blood vessel injury, refer to the published proceedings of the 1998 Beckman Conference.

**What is Homocyst(e)ine?**

Homocyst(e)ine, a thiol-containing amino acid, has the empirical formula $SHCH_2CH_2(NH_2)COOH$ and a molecular weight of 135.2. In vivo, the reactive sulfhydryl group (-SH) of homocyst(e)ine combines with the sulfhydryl group of cysteine to form a mixed disulfide and with the cysteine residues in proteins to form stable disulfide bonds. The auto-oxidized, dimeric form of homocyst(e)ine is referred to as homocystine. In the circulation, more than 90 percent of homocyst(e)ine is present in combination with plasma proteins, primarily albumin. Collectively, the sum of these species is referred to as total homocyst(e)ine and abbreviated tHcy.

**Screening for Homocystinuria**

Plasma homocystine measurements have been used to screen newborns for homocystinuria, an inborn error of homocyst(e)ine metabolism characterized by a marked increase in the concentrations of plasma and urinary tHcy. Homocystine, however, is unstable in plasma processed at room temperature and is rapidly converted to the more stable protein-bound forms. In vitro, deproteinization of plasma or serum must be done immediately after blood collection to detect free homocystine or the mixed disulfides in the supernatant fluid. Plasma or serum collected at room temperature with no deproteinization step is comprised entirely of protein-bound homocyst(e)ine. Using homocystine as a marker, a missed diagnosis of homocystinuria can result if deproteinization is not performed immediately.

The most effective screening method for diagnosis of homocystinuria is the measurement of total homocyst(e)ine in serum or plasma. In vitro, total homocyst(e)ine is stable with no special specimen preparation required, thereby avoiding the missed diagnosis of homocystinuria associated with a false-negative homocystine result. It is a matter of practical consideration that in the analytical measurement of tHcy, the initial step actually involves the reduction of all disulfide bonds to yield the free, reduced form of homocyst(e)ine.

**Source of Homocyst(e)ine in Humans**

Homocyst(e)ine is produced in the liver exclusively from the demethylation of dietary methionine, an essential amino acid found in red meats and dairy products. Unlike cholesterol, no homocyst(e)ine is produced in vivo starting with 2-carbon moieties. The interconversion of methionine and homocysteine depends on the availability of the methyl donor 5-methyltetrahydrofolate, cofactors vitamin $B_{12}$ and folate, and the enzyme activity of methionine synthase.

**How is Homocyst(e)ine Metabolized?**

After homocyst(e)ine is derived from methionine, it undergoes either remethylation to methionine or is metabolized all the way to sulfate via the intermediary compounds cystathionine and cysteine. Several B-complex vitamins serve as cofactors and substrates in the metabolism of methionine and homocyst(e)ine (Fig. 1). Folic acid and vitamin $B_{12}$ (cyanocobalamin) regulate metabolic pathways catalyzed by the enzymes methylenetetrahydrofolate reductase (MTHFR) and methionine synthase, whereas vitamin $B_6$ (pyridoxal phosphate) is a cofactor for cystathionine B-synthase (CBS). The latter route is referred to as the transsulfuration pathway. Individuals heterozygous for CBS deficiency are predisposed to hyperhomocyst(e)inemia and, although having a normal basal level of homocyst(e)ine, can be identified by the methionine loading test.

**What is a Normal Homocyst(e)ine Level?**

Using any of the standard analytical methods, values between 5 and 15 µmol/L are generally considered normal, albeit not optimal or desirable. Levels tend to increase with age and pre-menopausal women younger than 65 have lower values than men in the same age bracket. After menopause, women have comparable levels to men. Values <10 µmol/L may be considered desirable, values <12 µmol/L optimal, and values 12 to 15 µmol/L borderline. Moderate hyperhomocyst(e)inemia is defined as >15 to 30 µmol/L, intermediate as >30 to 100 µmol/L and severe as >100 µmol/L.

**What is the Relative Risk for Coronary Artery Disease (CAD)?**

Based on case data for 130 patients hospitalized with their first acute myocardial infarction (AMI) and 118 population controls, an odds ratio of 1.35 for each 3.0 µmol/L increase in homocyst(e)ine above 7.2 µmol/L can be used to calculate a relative risk for occlusive vascular disease. The calculation is:

$$\text{Risk of MI} = \frac{\text{tHcy} - 7.2}{3} \times 035 + 1.00$$

This study shows that even optimal levels of homocyst(e)ine (i.e., <12 µmol/L) pose an increased risk for arteriosclerosis compared with the lowest 20th percentile (<7.2 µmol/L) for population controls.

In another study involving 587 patients with confirmed CAD, Nygärd et al found a strong relationship between homocyst(e)ine levels and cardiovascular mortality rates. After 4.6 years, using 9.0 µmol/L as a reference point, the mortality rate increased 1.9-, 2.8- and 4.5-fold among patients with tHcy levels of 9.0-14.9, 15.0-19.9 and ≥ 20.0 µmol/L, respectively. This translates into a death rate of 3.6 percent for patients with a tHcy level <9.0 µmol/L compared with a mortality of 24.7 percent for those with levels >14.9 µmol/L.

Using homocyst(e)ine levels of <10 and >15 to compare relative risk for CAD mortality, Omenn et al suggested that the risk differential is comparable to that between total serum cholesterol levels of 189 and 275 mg/dL.

* * * *

**How Can Folate Deficiency be Differentiated From Vitamin $B_{12}$ Deficiency?**

For subclinical vitamin deficiencies, where vitamin $B_{12}$ and/or folate levels are normal or borderline low, a comprehensive metabolite profile has the highest diagnostic sensitivity for identifying patients at risk and for differentiating between suboptimal folate and vitamin $B_{12}$ status. A mega-

loblastic anemia profile consists of homocysteine, cystationine, methylmalonic acid and 2-methylcitrate.

In cobalamin deficiency, all four parameters are elevated, but in folate deficiency, typically only homocysteine and cystathionine are increased. Most importantly, elevated levels of these metabolites will correct to normal after treatment with the appropriate vitamin, but will not normalize after treatment with the wrong vitamin, even in pharmacological amounts. Serum metabolite levels can be rechecked five to 15 days after vitamin therapy is initiated.

* * * *

**Summary**

Homocyst(e)ine has been shown to be an independent risk factor for the development of atherosclerotic vascular disease. Acquired forms of hyperhomocyst(e)inemia are due to vitamin deficiencies and exposure to drugs and toxins. Familial homocystinuria is a homozygous expression of enzyme defects or deficiencies involving the Hcy metabolizing pathways.

The most important determinants of tHcy have been identified as age and sex, folate and vitamin $B_{12}$ status, and renal function. Vitamin supplementation, especially folic acid, is effective at lowering blood tHcy levels. High-risk patients, refractory to high-dose vitamin therapy, may benefit from tHcy-lowering drugs.

Normal levels of homocyst(e)ine are 5 µmol/L to 15 µmol/L Optimal levels, cautiously defined as <12 µmol/L, still pose some cardiovascular risk. Prevention programs target the lowering of tHcy levels to <10 µmol/L. The Nutrition Committee of the American Heart Association states: "A basal homocyst(e)ine level <10 µmol/L is a reasonable therapeutic goal for subjects at increased risk, defined as those patients with personal or family history of premature cardiovascular disease, as well as those with malnutrition, malabsorption syndromes, hypothyroidism, renal failure, systemic lupus erythematosus; those taking certain medications, eg, nicotinic add, theophylline, bile acid-

binding resins, methotrexate, and L-dopa: or those with recent nitrous oxide exposure.

It remains to be demonstrated in placebo-controlled multi-center clinical trials that a reduction of tHcy will lower morbidity and mortality both in patients with vascular disease and in the general population.

* * * *

**Plaintiffs' Exhibit 181**

**Megaloblastic Anemia Profiles:**
***Laboratory Screening and Confirmation***

By Peter W. Wentz, PhD, DABCC

[1999]

* * * *

**MEGALOBLASTIC ANEMIA PROFILE**

The megaloblastic anemia profile is comprised of methylmalonic acid, 2-methylcitrate, homocysteine and cystathionine. All four metabolites are elevated in most patients with cobalamin deficiency, whereas only homocysteine and cystathionine are elevated in most patients with folate deficiency.

- *Methylmalonic Acid (MMA).* The serum methylmalonic acid level is elevated in more than 95 percent of patients with clinically confirmed cobalamin deficiency. Values as high as 200,000 nmol/L have been observed, with a median value in the range of 3,500 nmol/L. Frequently an elevated serum MMA is seen before a low cobalamin develops in patients with pernicious anemia in remission, when maintenance vitamin $B_{12}$ injections are missed for several months.

The cutoff for MMA is set at +3sd above the mean for healthy blood bank donors. This level provides greater specificity for the diagnosis of cobalamin deficiency despite the loss of some sensitivity. For a confirmatory test, the highest diagnostic specificity is desirable. Moelby et al have concluded that serum methylmalonic acid is a sensitive and probably specific indicator of cobalamin deficiency, having a diagnostic efficiency of 95 percent.

Note that elevations of serum methylmalonic acid due to cobalamin deficiency do not respond to pharmacologic doses of folate. Using MMA value of >375 nmol/L as the reference standard for cobalamin deficiency, Holleland et al have

determined a diagnostic sensitivity of 40 percent and a diagnostic specificity of 98 percent for the serum cobalamin assay (cutoff of 230 pg/mL).

- *Homocysteine (HCy).* An elevated serum homocysteine level is a marker for both cobalamin and folate deficiencies. Values as high as 500 µmol/L have been observed in cobalamin deficiency, with a median of 70 µmol/L. In folate deficiency, folate values may be as high as 250 µmol/L, with a median of 50 µmol/L. Elevations of homocysteine due to folate deficiency do not respond to pharmacologic doses of cobalamin.

- *2-Methylcitrate.* Increased levels of propionyl-CoA lead to increased synthesis of 2-methylcitric acid by citrate synthase (Fig. 3), which usually uses acetyl-CoA instead of propionyl-CoA to form citric acid. In cobalamin deficiency, 2-methylcitrate values may be as high as 15,000 nmol/L. Serum levels of 2-methylcitrate are elevated in more than 85 percent of patients with clinically confirmed cobalamin deficiency. In folate deficiency, 2-methylcitrate is usually normal.

- *Cystathionine.* Homocysteine is metabolized to cystationine by the transsulfuration pathway (Fig. 2b) and in cases of cobalamin deficiency cystathionine values may be as high as 4,000 nmol/L. In folate deficiency, levels may be as high as 18,000 nmol/L. Cystationine is shown to be elevated in 87 percent and 95 percent of patients with cobalamin and folate deficiency, respectively.

Serum levels of these metabolites are useful in diagnosing and distinguishing between cobalamin and folate deficiency. Savage et al concluded that normal levels of both methylmalonic acid and homocysteine rule out clinically significant cobalamin deficiency with virtual certainty. Furthermore, an elevated methylmalonic acid (or both MMA and Hcy) is much more specific for cobalamin deficiency than is a low cobalamin level, which is frequently seen without any other clinical evidence of deficiency. The only significant limitation to the specificity of an elevated MMA or a combined elevated MMA/Hcy is underlying renal insufficiency or

volume depletion, which may falsely elevate both metabolites.

Statistically, both MMA and Hcy are elevated in approximately 98 percent of cobalamin-deficient patients with megaloblastic anemia, whereas methylmalonic acid is more often elevated than homocysteine in non-anemic patients. Nevertheless, methylmalonic acid was normal in seven of 434 episodes of cobalamin deficiency, although homocysteine was elevated in six of the seven episodes. They recommend combining the two metabolite determinations to improve the rate of correctly diagnosing cobalamin deficiency over that of methylmalonic acid alone.

In this same study. Savage et al found an elevated homocysteine in 97.5 percent of 121 episodes of folate deficiency, compared with only a 12.4 percent (15/121) incidence of elevated methylmalonic acid. Fourteen out of 15 patients with elevated methylmalonic acids had renal dysfunction. The one exception could not be explained and is presumably a false-positive result.

When used together with serum levels of cobalamin and folate, it's possible to diagnose and distinguish between cobalamin and folate deficiency in the vast majority of patients. The combined approach to diagnosis is important because approximately 5 percent of patients with clinically confirmed cobalamin or folate deficiency will have normal serum metabolite levels, and as many as 10 percent will have values for serum cobalamin and folate that are well within the normal range.

* * * *

**Plaintiffs' Exhibit 182**

* * * *

## *Health Care Providers*

**About LabCorp**

LabCorp is one of the largest clinical laboratory networks in the United States, with service extended to more than 100,000 clients in 49 states. Through a comprehensive menu of clinical test options, from the clinical chemistry to sophisticated technologies such as polymerase chain reaction (PCR) and DNA probe analyses, LabCorp assists physicians and other health care practitioners in the diagnosis, treatment, and monitoring of their patients.

**About this site**

LabCorp regularly publishes literature on several areas of clinical laboratory testing. This website is designed to enable health care providers to find the information they need to better serve their patients. The section is divided into two subsections, with applicable subtopics for each, as listed below.

**Medical Specialties**

- Allergy
- Cardiology
- Endocrinology
- Gastroenterology
- Geriatrics
- Infectious Disease
- Nephrology
- Oncology
- Pulmonology
- Rheumatology

**Surgical Specialties**

- Obstetrics/Gynecology
- Orthopaedics
- Urology

Select the appropriate link below for access to specialized LabCorp publications as well as links to other informative sites. Publications, links, and other communications found on this site are not intended as a substitute for consultation with an appropriate technical expert.

Medical Specialties Surgical Specialties
Patient Service Centers Locator

**LabCorp®**
**358 South Main Street**
**Burlington, NC 27215**
**336-222-7566**

8/14/01

**Plaintiffs' Exhibit 183**

## Homocyst(e)ine, Plasma or Serum (706994)

**CPT 82131**

**Related Information**

- Anemia Profile, Megaloblastic, Serum

* * * *

**Reference Interval**

Homocyst(e)ine:

- Normal: 5-15 µmol/L
- Optimal: <12 µmol/L

Hyperhomocyst(e)inemia:

- Borderline: 12-15 µmol/L
- Moderate: >15-30 µmol/L
- Intermediate: >30-100 µmol/L
- Severe: >100 µmol/L

* * * *

Studies have shown that even optimal levels of homocyst(e)ine pose an increased risk for arteriosclerosis compared with the lowest 20th percentile (<7.2 µmol/L) of population controls.

**Use:** Evaluate borderline folate deficiency as a part of Anemia Profile, Megaloblastic, Serum (test 706960). Homocysteine has been shown to be an independent risk factor for the premature development of coronary artery disease and thrombosis.

* * * *

**References**

Fortin LJ and Genest J Jr, "Measurement of Homocyst(e)ine in the Prediction of Arteriosclerosis," *Clin Biochem*, 1995, 28(2):155-62 (review).

Kang SS, Wong PW, and Malinow MR, "Hyperhomocyst(e)inemia as a Risk Factor for Occlusive Vascular Disease," *Ann Rev Nutr*, 1992, 12:279-98.

Malinow MR, "Plasma Homocyst(e)ine and Arterial Occlusive Diseases: A Mini Review," *Clin Chem*, 1995, 41(1):173-6.

Robinson K, Mayer, E, and Jacobsen DW, "Homocysteine and Coronary Artery Disease," *Cleveland Clin J Med*, 1994 61(6):438-50.

Ueland PM, Refsum H, Stabler SP, et al, "Total Homocysteine in Plasma or Serum: Methods and Clinical Applications, *Clin Chem*, 1993, 39(9):1764-79.

Verhoef P, Stampfer MJ, Buring JE, et al, "Homocysteine Metabolism and Risk of Myocardial Infarction: Relation With Vitamins $B_6$, $B_{12}$, and Folate," *Am J Epidemiol*, 1996, 143(9):845-59.

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**Plaintiffs' Exhibit 184**

## Anemia Profile, Megaloblastic, Serum (706960)

* * * *

**Reference Interval** See table.

**Megaloblastic Anemia Profile, Serum**

| Serum Metabolite | Normal Range | % High Values | $B_{12}$ Deficiency Range | % High Values | Folate Deficiency Range | % High Values |
|---|---|---|---|---|---|---|
| Methylmalonic acid | 73-271 | <3 | 272-200,000 | >95 | 73-271 nmol/L | <3 |
| 2-Methylcitric acid | 60-228 | <3 | 228-15,000 | >80 | 60-228 nmol/L | <3 |
| Homocysteine | 5.1-13.9 | <3 | 14-500 | >95 | 14-250 µmol/L | >95 |
| Cystathionine | 44-342 | <3 | 342-4000 | >80 | 342-18,000 nmol/L | >80 |

Serum methylmalonic acid and homocysteine are the primary metabolic tests for diagnosing and distinguishing between $B_{12}$ and folate deficiency. They can be used in conjunction with the serum $B_{12}$, which is usually low or low normal (<350 pg/mL) in $B_{12}$ deficiency, and the serum folate, which is usually low or low normal (<5mg/mL) in folate deficiency. 2-Methylcitric acid and cystathionine provide confirmatory evidence for such deficiencies. Homocysteine and especially cystathionine may also be high in $B_6$ deficiency.

Elevated levels of serum metabolites will correct to normal after treatment with the appropriate vitamin but will not correct after treatment with the wrong vitamin, even in pharmacologic amounts.

* * * *

**Additional Information** The megaloblastic anemia profile monitors four compounds: methylmalonic acid, 2-methylcitric acid, homocysteine, and cystathionine. It has been shown that all four of these compounds are elevated if a

patient is suffering from vitamin $B_{12}$ deficiency (megaloblastic anemia, cobalamin deficiency) and that homocysteine and cystathionine are elevated if there is a folic acid deficiency. Methylmalonic acid and homocysteine are the primary tests for diagnosis. 2-Methylcitric acid and cystathionine provide confirmatory information.

This method is a more specific diagnostic tool for $B_{12}$ and folate deficiencies as it has been shown that some patients with deficiencies will have normal or borderline serum $B_{12}$ or folate. These deficiencies can lead to incapacitating and life-threatening neuropsychiatric abnormalities which are completely reversible by treatment with $B_{12}$ or folic acid. As with any such condition early detection is very important. This profile provides early detection and differentiation. This differentiation is very important because both $B_{12}$ and folate deficiencies have indistinguishable hematological abnormalities. The greatest improvement is observed when the proper vitamin is used and only $B_{12}$ will correct the neuropsychiatric abnormalities seen in cobalamin deficiency. There is also recent literature linking cardiovascular disease with homocysteine elevation.

**References**

Lindebaum MD, Healton EB, et al, "Neuropsychiatric Disorders Caused by Cobalamin Deficiency in the Absence of Anemia or Macrocytosis," *N Engl J Med*, 1988, 318:1720-8.

Marcell PD, Stabler SP, et al, "Quantitation of Methylmalonic Acid and Other Dicarboxylic Acids in Normal Serum and Urine Using Capillary Gas Chromatography-Mass Spectrometry," *Anal Biochem*, 1985, 150(1):58-66.

Stabler SP, Marcell PD, Podell ER, et al, "Elevation of Total Homocysteine in the Serum of Patients With Cobalamin or Folate Deficiency Detected by Capillary Gas Chromatography-Mass Spectrometry," *J Clin Invest*, 1988, 81(2):466-74.

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http://www.labcorp.com/datasets/labcorp/html/chapter/mono/
tx008700.htm 7/19/99

**Plaintiffs' Exhibit 185**

# Total Homocysteine in Plasma or Serum: Methods and Clinical Applications

*Clinical Chemistry, Vol. 39, No. 9, [1764-1779]*

*1993*

Per M. Ueland,[1,5] Helga Refsum,[1] Sally P. Stabler,[2] M. Rene Malinow,[3] Anders Andersson,[4] and Robert H. Allen[2]

Total homocysteine is defined as the sum of all homocysteine species in plasma/serum, including free and protein-bound forms. In the present review, we compare and evaluate several techniques for the determination of total homocysteine. Because these assays include the conversion of all forms into a single species by reduction, the redistribution between free and protein-bound homocysteine through disulfide interchange does not affect the results, and total homocysteine can be measured in stored samples.

* * * *

*Reduction.* Determination of total Hcy in plasma/serum requires the reduction of the disulfide bond between Hcy and other thiols or albumin.

* * * *

**Plaintiffs' Exhibit 194**

**LabCorp Directory of Services & Interpretive Guide [1999]**

* * * *

**ANEMIA PROFILE, MEGALOBLASTIC, SERUM .....706960**

**CPT 82131 (x4)**

**Related Information**

Methylmalonic Acid, Serum *on page 290*
Methylmalonic Acid, Urine *on page 290*
Vitamin $B_{12}$ *on page 385*

**Synonyms** Megaloblastic Anemia, Serum

**Test Includes** Cystathionine; homocysteine; 2-methylcitric acid; methylmalonic acid

**Specimen** Serum or plasma

**Volume** 2.2 mL

**Minimum Volume** 1.1 mL

**Container** Red stopper tube or serum-separator tube or green-stopper (heparin plasma) tube

**Collection** Separate serum or plasma from cells within 1 hour of collection and transfer to plastic transport tube.

**Storage Instructions** Refrigerate

**Causes for Rejection** Specimen received not separated from cells, plasma from a light blue-stoppered or yellow-stoppered tube; citrate interferes with assay; homocysteine results increase by approximately 35% and 75% for specimens not centrifuged and/or not separated from the clot for periods of 4 and 24 hours respectively

**Reference Interval** See table

**Megaloblastic Anemia Profile, Serum**

| Serum Metabolite | Normal Range | % High Values | $B_{12}$ Deficiency Range | % High Values | Folate Deficiency Range | % High Values |
|---|---|---|---|---|---|---|
| Methylmalonic acid | 73-271 | <3 | 272-200,000 | >95 | 73-271 nmol/L | <3 |
| 2-Methylcitric acid | 80-228 | <3 | 228-15,000 | >90 | 60-228 nmol/L | <3 |
| Homocysteine | 51-139 | <3 | 14-500 | >95 | 14-250 µmol/L | >95 |
| Cystathionine | 44-342 | <3 | 342-4000 | >80 | 342-18,000 nmol/L | >80 |

Serum methylmalonic acid and homocysteine are the primary metabolic tests for diagnosing and distinguishing between $B_{12}$ and folate deficiency. They can be used in conjunction with the serum $B_{12}$, which is usually low or low normal (<350 pg/mL) in $B_{12}$ deficiency, and the serum folate, which is usually low or low normal (<5 mg/mL) in folate deficiency. 2-Methylcitric acid and cystathionine provide confirmatory evidence for such deficiencies. Homocysteine and especially cystathionine may also be high in $B_{12}$ deficiency.

Elevated levels of serum metabolites will correct to normal after treatment with the appropriate vitamin but will not correct after treatment with the wrong vitamin, even in pharmacologic amounts.

Any of the four metabolites can be elevated due to renal insufficiency or intravascular volume depletion. This occurs most commonly in the case of 2-methylcitric acid and cystathionine. Elevated metabolite levels do not correct with $B_{12}$, folate or $B_6$ treatment unless vitamin deficiency coexists.

Serum metabolite levels can be rechecked 5-15 days after vitamin therapy.

Normal ranges 6 hours postoral methionine load (100 mg L-methionine/kg body weight) are as follows: homocysteine 16.5-45.7 µmol/L and cystathionine: 424-2500 nmol/L. Methylmalonic acid and 2-methylcitric acid do not change after a methionine load.

**Use** Diagnose megaloblastic anemia. Elevated levels of methylmalonic acid provide an early indication of cobalamin ($B_{12}$) deficiency.

**Methodology**–Gas chromatography/mass spectrometry (GC/MS)

**Additional Information** The megaloblastic anemia profile monitors four compounds: methylmalonic acid, 2-methylcitric acid, homocysteine, and cystathionine. It has been shown that all four of these compounds are elevated if a patient is suffering from vitamin $B_{12}$ deficiency (megaloblastic anemia, cobalamin deficiency) and that homocysteine and cystathionine are elevated if there is a folic acid deficiency. Methylmalonic acid and homocysteine are the primary tests for diagnosis. 2-Methylcitric acid and cystathionine provide confirmatory information.

This method is a more specific diagnostic tool for $B_{12}$ and folate deficiencies as it has been shown that some patients with deficiencies will have normal or borderline serum $B_{12}$ or folate. These deficiencies can lead to incapacitating and life-threatening neuropsychiatric abnormalities which are completely reversible by treatment with $B_{12}$ or folic acid. As with any such condition early detection is very important. This profile provides early detection and differentiation. This differentiation is very important because both $B_{12}$ and folate deficiencies have indistinguishable hematological abnormalities. The greatest improvement is observed when the proper vitamin is used and only $B_{12}$ will correct the neuropsychiatric abnormalities seen in cobalamin deficiency. There is also recent literature linking cardiovascular disease with homocysteine elevation.

* * * *

## HOMOCYST(E)INE, PLASMA OR SERUM .....706994

**CPT 82131**

**Related Information**

Anemia Profile, Megaloblastic Serum *on page 114*

**Specimen** Plasma or serum

**Volume** 2.2 mL

**Minimum Volume** 1.1 mL

**Container** Lavender-stopper (EDTA) tube or red-stopper tube or serum-separator tube or green-stopper (heparinized plasma) tube.

**Collection** Separate plasma or serum from cells within 1 hour of collection and transfer to plastic transport tube

**Storage Instructions** Refrigerate

**Patient Preparation** A fasting specimen is preferred to establish baseline values or monitor treatment.

**Causes for Rejection** Specimen received not separated from cells; plasma from a light blue-stopper or yellow-stopper tube; citrate interferes with assay. Homocysteine results increase by approximately 35% and 75% for specimens not centrifuged and/or not separated from the clot for periods of 4 and 24 hours, respectively.

**Reference Interval**

Homocyst(e)ine:

- Normal: 5-15 µmol/L
- Optimal: < 12 µmol/L

Hyperhomocyst(e)inemia:

- Borderline: 12-15 µmol/L
- Moderate: > 15-30 µmol/L
- Severe > 100 µmol/L

Detection limit: 2.5 µmol/L

Studies have shown that even optimal levels of homocysteine pose an increased risk for arteriosclerosis compared with the lowest 20th percentile (< 7.2 µmol/L) of population controls.

**Use** Evaluate borderline folate deficiency as a part of Anemia Profile. Megaloblastic Serum (test 706960) Homocysteine has been shown to be an independent risk factor for the premature development of coronary artery disease and thrombosis.

**Methodology** Immunoassay

* * * *

**Plaintiffs' Exhibit 195**

**LabCorp Directory of Services & Interpretive Guide**

* * * *

**HOMOCYST(E)INE, PLASMA OR SERUM .....706994**

**CPT 82131**

**Specimen** Plasma or serum.

**Volume** 1 mL

**Minimum Volume** 0.3 mL

**Container** Lavender-stopper (EDTA) tube or red-stopper tube or serum-separator tube or green-stopper (heparinized plasma) tube

**Collection** EDTA plasma is the preferred specimen. Separate plasma from cells immediately to avoid a false elevation of homocysteine. After 1 hour at room temperature, a 10% increase may be seen. Transfer plasma or serum to a plastic transport tube.

**Storage Instructions** Refrigerate

**Patient Preparation** A fasting specimen is preferred to establish baseline values or monitor treatment.

**Causes for Rejection** Specimen received not separated from cells; plasma from a light blue-stopper or yellow-stopper tube; citrate interferes with assay. Homocysteine results increase by approximately 35% and 75% for specimens not centrifuged and/or not separated from the clot for periods of 4 and 24 hours, respectively.

**Reference Interval**

Homocyst(e)ine:

- normal: 5-15 µmol/L
- desirable: < 10 µmol/L

Hyperhomocyst(e)inemia:

- borderline: 12-15 µmol/L
- moderate: > 15-30 µmol/L
- intermediate: > 30-100 µmol/L
- severe: > 100 µmol/L

Studies have shown that even optimal levels of homocyst(e)ine pose an increased risk for arteriosclerosis compared with the lowest 20th percentile (< 7.2 µmol/L) of population controls.

**Use** Homocysteine has been shown to be an independent risk factor for the premature development of coronary artery disease and thrombosis. This test is intended for use in screening patients who may be at risk for heart disease and stroke. Evaluate borderline folate deficiency as a part of Anemia Profile, Megaloblastic, Serum (test 706960).

**Methodology** Immunoassay

* * * *

**Plaintiffs' Exhibit 218**



**From:** Peter Wentz -
**To:** mml@mayo.edu
**Date:** Thu, Jun 10, 1999 4:13 PM
**Subject:** Homocysteine, Plasma (#80379)

Dear Sir,

Is this test performed pursuant to a licensing agreement with Competitive Technologies (Patent Number: 4,940,658)? Under Claim 13 of the patent the methodology of the homocysteine assay is unimportant. Claim 13 claims any method for detecting a deficiency of either cobalamin or folate by assaying for homocysteine. Have you been able to successfully challenge this provision? Thanks for the feedback.

Regards,

Peter Wentz

**Plaintiffs' Exhibit 278**

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF COLORADO

Civil Action No. 99-Z-870

METABOLITE LABORATORIES, INC.,
a Colorado corporation, and
COMPETITIVE TECHNOLOGIES, INC.,
a Delaware corporation,

Plaintiffs,

vs.

LABORATORY CORPORATION OF AMERICA HOLDINGS d/b/a LabCorp, a Delaware corporation,

Defendant.

**STIPULATION**

Based upon a review of available data, Plaintiffs Metabolite Laboratories, Inc. ("Metabolite") and Competitive Technologies, Inc. ("CTI") and Defendant Laboratory Corporation of America Holdings d/b/a LabCorp ("LabCorp") hereby stipulate as follows:

1. The total number of single homocysteine tests (Test # 706994) performed by LabCorp using the Abbott method and billed by LabCorp (from August 1, 1998 to September 30, 2001 for the LCBS System, from August 1, 1998 to September 30, 2001 for the BDS System, from August 1, 1998 to October 29, 2001 for the Dallas Lab and from August 1, 1998 to October 31, 2001 for the San Diego Lab) was 351,458 tests.

2. The billed revenues associated with the 351,458 single homocysteine tests (Test #706994) performed by LabCorp

using the Abbott method from August 1, 1998 to the dates indicated in Stipulation No. 1 were $16,989,416.81.

This 13th day of November, 2001.

/s/ J. Gregory Whitehair
Glenn K. Beaton
J. Gregory Whitehair
GIBSON, DUNN
& CRUTCHER LLP
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Attorneys for Defendant
Laboratory Corporation of
America Holdings

## Defendant's Exhibit K

[SEAL]

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

| SERIAL NUMBER | FILING DATE | FIRST NAMED APPLICANT | | ATTORNEY DOCKET NO. |
|---|---|---|---|---|
| 06/933,553 | 11/20/86 | ALLEN | R | CU280/281 |

BARBARA A. SHIMEI
UNIVERSITY PATENTS, INC.
P.O. BOX 901
WESTPORT, CT 06881

| EXAMINER | |
|---|---|
| SCHEINER, T | |
| ART UNIT | PAPER NUMBER |
| 182 | 4 |

DATE MAILED

08/01/88

This is a communication from the examiner in charge of your examination.
COMMISSIONER OF PATENTS AND TRADEMARKS

Part II SUMMARY OF ACTION

* * * *

1. ☒ Claims 1-32 are pending in the application.

* * * *

4. ☒ Claims 1-32 are rejected.

* * * *

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103.

This application has been filed with informal drawings which are acceptable for examination purposes only. Formal drawings will be required when the application is allowed.

The Abstract is objected to because it should be entitled "Abstract of the Disclosure".

Claims 1-32 are rejected under 35 U.S.C. 112, first and second paragraphs, as the claimed invention is not described in such full, clear, concise and exact terms as to enable any person skilled in the art to make and use the same, and/or for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claim 1, the word "different" is superfluous and should be removed. In step (a), "the" should be replaced with "a", "an known" should be "known".

Claim 2 is vague and indefinite in its recitation of "appropriate derivatives" as no criteria of "appropriateness" are provided.

Claims 6 and 9 are vague and indefinite in their recitations of "partially purifying" since no degree or method of purification is mentioned.

In claim 10, the language "an appropriate non-sulfhydryl amino acid internal. . ." is indefinite, the claim should indicate that the standard is a labeled derivative of the amino acid of interest.

Claim 13 should recite discrete, sequential process steps, for example, obtaining a sample, contacting the sample, etc. The final step should be clearly related to the preamble of the claim.

Claims 13-22 are vague and indefinite in their recitations of "elevated levels" and "normal levels" of total homocysteine. The claims should include quantitative language.

Claim 17 is vague and indefinite in its recitation of "appropriate detection means".

Claim 18 should also recite discrete, sequential process steps, concluding with a step clearly related to the preamble.

The language "and/or" in claims 18 and 19 is confusing. The claims should clearly state each of the possible deficiencies or combinations of deficiencies in the preamble.

Claim 24 is vague and indefinite in its recitation of "appropriate" derivatives.

The language "partially purifying" in claim 28 is indefinite.

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) and (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 1-12 are rejected under 35 U.S.C. 103 as being unpatentable over Laseter et al and Freed et al.

Amino acid derivatives, including silyl derivatives, are conventionally determined by mass spectrometry, for an example, see Laseter et al (lines 1-3, and last 5 lines). Further, as shown by Freed et al, the use of radiolabeled derivatives or analogs of compounds of interest as internal reference standards is well known in the art. It would have been obvious for one of ordinary skill in the art to have determined the amount of any particular amino acid or amino acid derivative by mass spectrometry, as taught by Laseter et

al, by combining it with an internal reference standard (a known amount of labeled derivative or analog of the compound of interest) and determining the ratio of labeled to unlabeled compound, as taught by Freed et al.

Claims 13-32 are rejected under 35 U.S.C. 103 as being unpatentable over Laseter et al and Freed et al as applied to claims 1-12 above, and further in view of Mudd et al and Frenkel et al.

Mudd et al show that elevated blood and urine levels of homocysteine and methylmalonic acid are associated with a deficiency in coenzymatically active vitamin $B_{12}$ derivatives, even when total vitamin $B_{12}$ levels are normal. Frenkel et al teach that elevated levels of methylmalonic acid are associated with cobalamin deficiency and that methylmalonic aciduria can reveal those instances where a cobalamin deficiency exists, but serum cobalamin levels are normal. It would have been obvious for one of ordinary skill in the art to determine cobalamin "or" folate deficiency indirectly by measuring homocysteine and methylmalonate levels, since it is known that direct methods may yield misleading results. With reference to claims 29-32, it is conventional in the art to relieve the symptoms of cobalamin or folate deficiency, regardless of the method of diagnosis, by administering cobalamin or folate.

Any inquiry concerning this communication should be directed to T. Sheiner at telephone number 703-557-8175.

/s/ TRS

SCHEINER : ew

7-14-88

retyped 7-15-88

/s/ Robert J. Warden
Robert J. Warden
Supervisory Patent Examiner
Art Unit 182

**Defendant's Exhibit M**

IN THE UNITED STATES PATENT
AND TRADEMARK OFFICE

In re Application of:
Robert H. Allen
Sally P. Stabler
John Lindenbaum

Serial No. 933,553 Group Art Unit: 182

Filed: 20 November 1986 Examiner: T. Scheiner

For: Assay for Sulfhydryl Amino Acids and Methods for Detecting and Distinguishing Cobalamin and Folic Acid Deficiency

February 1989

Commissioner of Patents and Trademarks
Washington, DC 20231

Sir:

REQUEST FOR EXTENSION OF TIME

Applicants respectfully request that the period for responding to the Official Action of 1 August 1988 be extended for three (3) months so as to expire on 1 February 1989.

The Commissioner is hereby authorized to charge the necessary fees due under 37 CFR 1.17(c) to Deposit Account No. 21-0691. A duplicate copy of this sheet is enclosed.

AMENDMENT

* * * *

Rejection under 35 U.S.C. 112:

* * * *

Claim 13 should recite discrete, sequential process steps, for example, obtaining a sample, contacting the sample, etc. The final step should be clearly related to the preamble of the claim.

As Applicants are the first to detect cobalamin or folate deficiency by assaying body fluids for total homocysteine, it is believed that they are entitled to a claim of equivalent scope, not limited to any particular process steps or methods.

Claims 13-22 are vague and indefinite in their recitations of "elevated levels" and "normal levels" of total homocysteine The claims should include quantitative language.

As shown on page 18, first full paragraph, the normal range for homocysteine in human serum at the time this invention was made was understood to be 7-22 μmol/liter and in human urine was 1-20 μmol/liter. Higher (elevated) levels of homocysteine are indicative of cobalamin and/or folate deficiency. Methods for determining such ranges for different body tissues are well-known in the art. The claims are fully supported by the specification and Applicants see no reason why the claims should be limited to particular ranges.

* * * *

Rejection of Claims 13-21 under 35 USC 103:

Claims 13-32 are rejected under 35 U.S.C. 103 as being unpatentable over Laseter et al and Freed et al as applied to claims 1-12 above, and further in view of Mudd et al and Frenkel et al.

Mudd et al show that elevated blood and urine levels of homocysteine and methylmalonic acid are associated with a deficiency in coenzymatically active vitamin B12 derivatives, even when total vitamin B12 levels are normal.

Frenkel et al teach that elevated levels of methylmalonic acid are associated with cobalamin deficiency and that methylmalonic aciduria can reveal those instances where a cobalamin deficiency exists, but serum cobalamin levels are normal. It would have been obvious for one of ordinary skill in the art to determine cobalamin "or" folate deficiency indirectly by measuring homocysteine and methylmalonate levels, since it is known that direct methods may yield misleading results. With reference to claims 29-32, it is conventional in the art to relieve the symptoms of cobalamin or folate deficiency, regardless of the method of diagnosis, by administering cobalamin or folate.

Claim 13 concerns a method for detecting a deficiency of cobalamin or folate in warm-blooded animals by assaying body fluids for the presence of elevated levels of total homocysteine.

Claim 14 is similar to Claim 13 but it is specified that said assay is performed according to the method of Claim 7.

Claim 15 depends from Claim 13 and specifies that said assay comprises subjecting the sample to chromatography.

Claim 16 depends from Claim 15 and specifies that said chromatography is high performance liquid chromatography with an electrochemical detector set to respond to thiol groups.

Claim 17 depends from Claim 13 and specifies that the assay for the presence of elevated levels of total homocysteine comprises converting homocysteine present in the sample to labelled S-adenosylhomocysteine by exposure to S-adenosylhomocysteine hydrolase and radioactive S-adenosine, and quantifying the labelled S-adenosylhomocysteine.

Claim 18 concerns a method for detecting a deficiency of cobalamin, folate, or both in warm-blooded animals and distinguishing therebetween, comprising assaying body fluids for the presence of elevated levels of total homocysteine and methylmalonic acid, wherein normal levels of total homocysteine indicate no cobalamin or folic acid deficiency,

elevated levels of both total homocysteine and methylmalonic acid indicate cobalamin deficiency, and elevated levels of total homocysteine combined with normal levels of methylmalonic acid indicate folic acid deficiency.

Claim 19 is similar to Claim 18 except that said assay for total homocysteine is specifically performed according to the method of Claim 7.

Claim 20 depends from Claim 18 and specifies that said assay for total homocysteine is done via chromatography.

Claim 21 depends from Claim 20 and specifies that said chromatography is high performance liquid chromatography with an electro-chemical detector set to respond to thiol groups.

Claim 22 depends from Claim 18 and specifies that said assay for total homocysteine is done by converting any homocysteine present in the sample to labelled S-adenosylhomocysteine by exposure to S-adenosylhomocysteine hydrolase and radioactively labelled adenosine.

Claim 23 depends from Claim 18 and as amended specifies that the methylmalonic acid is assayed by: a) combining the tissue sample with an internal reference standard comprising a known amount of methylmalonic acid labelled with a stable isotope marker; b) measuring the relative amounts of labelled and unlabelled methylmalonic acid present with a mass spectrometer; c) calculating the ratio of labelled to unlabelled methylmalonic acid present; and d) deriving the amount of unlabelled methylmalonic acid present in the sample. The claim has been amended to clarify that relative amounts are measured in step (b), which are then quantified in step (d).

Claim 24 depends from Claim 23 and adds the step of derivatizing the labelled and unlabelled methylmalonic acid after addition of the internal standard and before measurement on the mass spectrometer.

Claim 25 depends from Claim 24 and specifies that the derivatives are silyl derivatives.

Claim 26 depends from Claim 25 and specifies that the silyl derivatives are obtained by exposing the methylmalonic acid to N-methyl-N-(t-butyldimethylsilyl) trifluoroacetamide.

Claim 27 depends from Claim 23 or 24 and specifies that the internal reference standard comprises deuterated methylmalonic acid.

Claim 28 depends from Claim 26 and adds the step of partially purifying the methylmalonic acid after step (a) and before step (b).

Claim 29 as amended concerns a method of treating humans for cobalamin deficiency, wherein at least one body tissue from said human is assayed for the presence of cobalamin deficiency according to the method of Claim 18, and thereafter sufficient cobalamin is administered to said human to return the total homocysteine levels to normal.

Claim 30 is similar to Claim 29 except that the cobalamin deficiency is assayed for by the method of Claim 18.

Claim 31 as amended concerns a method of treating humans for folic acid deficiency which comprises assaying for the presence of folic acid deficiency according to the method of Claim 13, and administering to the human enough folic acid to return the total homocysteine levels to normal.

Claim 32 is similar to Claim 31 except that the folic acid levels are assayed via the method of Claim 18.

The heart of these claims is the concept that total homocysteine is elevated in patients with cobalamin and folic acid deficiency, while methylmalonic acid values are elevated only in patients with cobalamin deficiency. The correlative values can be charted as follows:

| | Total Homocysteine | Methylmalonic acid |
|---|---|---|
| Cobalamin deficiency | + | + |
| Folic acid deficiency | + | Normal |

wherein "+" indicates an elevated level. It was not previously known that elevated levels of homocysteine correlate with cobalamin and folate deficiency, nor was it previously known or suggested to use a combination of assays for total homocysteine and methylmalonic acid to diagnose and distinguish between these deficiencies.

* * * *

Mudd, S.H., H.L. Levy, and R.H. Abeles, Chem. Abstr. (1969) 70:150 (Abstr. No. 113151p) [hereinafter Mudd] discloses high blood and urine levels of homocysteine and cystathionine and a decrease in methionine in a fatally ill infant, attributed to an inability to accumulate coenzymatically active vitamin B12. Total vitamin B12 levels were normal, suggesting that the defect was in metabolism rather than transport.

The Examiner is suggesting that because homocysteine was elevated in an infant with an inborn error in B12 metabolism, it would be obvious to assay everyone who is cobalamin deficient for elevated levels of homocysteine. This logic is erroneous. Since B12 levels were normal, it is clear that this infant was not suffering from B12 deficiency. There is no teaching that high levels of total homocysteine in such genetically impaired infants correlates with cobalamin deficiency in normal humans.

Dr. Mudd himself, in a book published in 1974 (five years after the above-cited work) stated:

> . . . Insufficient data are available to establish whether homocystinuria is a rare or common manifestation in untreated patients with selective genetic malabsorption of B12. If it is common, the question arises as to whether patients with B12 deficiency for other reasons are homocystinuric. Patients with untreated pernicious anemia [the most common cause of cobalamin deficiency] in some cases have mild non-specific aminoaciduria but homocystinuria has not been mentioned. . . . B12-

> deficient pigs have . . . an abnormal elevation of serum homocysteine-cysteine mixed disulfide. It would be worthwhile to reexamine with modern sensitive methods the plasma of B12-deficient patients for such changes, and their urine for possible homocystinuria. Such studies would also be of interest in patients with malabsorption of vitamin B12 due to genetically determined absence of intrinsic factor activity or with genetic malabsorption of foic acid. [citations omitted, comment added]

Mudd, S.H. in Heritable Disorders of Amino Acid Metabolism: Patterns of Clinical Expression and Genetic Variation, W.L. Nyhan, ed. (John Wiley & Sons, New York, 1974), pp.436-437 [copy enclosed for the convenience of the Examiner].

Thus, even five years after the referenced work, Mudd is not sure whether homocystinuria is commonly associated with genetic malabsorption of B12 or is ever seen in other causes of cobalamin deficieny, including the most common, pernicious anemia. While Mudd suggests it would be interesting to examine the urine for elevated levels of homocysteine, he is by no means teaching that such a correlation exists. It was not until the work of the present inventors that the correlation was tested and proven.

* * * *

## Defendant's Exhibit P

[SEAL]

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

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| 06/933,553 | 11/20/86 | ALLEN | R | CU280/281 |
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BARBARA A. SHIMEI
UNIVERSITY PATENTS, INC.
P.O. BOX 901
WESTPORT, CT 06881

| EXAMINER | |
|---|---|
| SCHEINER, T | |
| ART UNIT | PAPER NUMBER |
| 182 | 4 |

DATE MAILED:

05/26/89

* * * *

Part II SUMMARY OF ACTION

1. ☒ Claims 1-32 are pending in the application.

Of the above, claims _____ are withdrawn from consideration.

2. ☐ Claims _____ have been cancelled.

3. ☒ Claims 1-12, 14 and 18-28 are free of the prior art of record.

4. ☒ Claims 13, 15-17 and 29-32 are rejected.

* * * *

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless-

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 13 and 15-17 are rejected under 35 U.S.C. 102a or b as being anticipated by Applicants' admitted state of the prior art.

In the absence of a correlation step, the preamble of claim 13 merely recites an intended use of the invention. The claim lacks a positive limitation for correlating to a particular condition and has only one method step recited. Applicants admit on pages 12 and 13 of the specification that assays for homocysteine are known. With respect to claims 15-17, applicants further admit that measurement of homocysteine by HPLC is conventional (R. Saetre et al, 1978), as is measurement of homocystein converted to S-adenosylhomocysteine (H. Refsum et al, 1985).

The text of those sections of Title 35 U. S. Code not included in this action can be found in a prior Office action.

Claims 29-32 are rejected under 35 U.S.C. 103 as being unpatentable over Applicants admitted state of the prior art.

On page 3 of the specification, and numerous other locations, applicants admit that treatment of diagnosed cobalamin or folic acid deficiencies by administration of cobalamin or folic acid, respectively, is conventional in the art. It would have been obvious for one of ordinary skill in the art to relieve the symptoms of cobalamin or folate deficiency, regardless of the method of diagnosis, by administering cobalamin or folic acid. In corporation of the instant method of diagnosis into an old treatment protocol does not provide unexpected results and cannot be considered unobvious (In re Durden, Jr. et al 226 USPQ 359).

R. Saetre et al and H. Refsum et al, cited on page 12 of the specification are unavailable to the examiner. It is requested that Applicant supply these pertinent references in order to comply with 37 CFR 1.56.

Claims 1-12. 14 and 18 are free of the prior art of record.

The references kindly supplied by Applicants in their Information Disclosure Statement of April 20, 1989 have been considered, however, a listing of the references on PTO form 1449 to complete the record would be greatly appreciated.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Toni R. Scheiner whose telephone number is (703) 557-8175.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 557-0664.

Scheiner-fg

5/24/89

/s/ TRS

/s/ Robert J. Warden
Robert J. Warden
Supervisory Patent Examiner
Art Unit 182

**Defendant's Exhibit R**

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | |
|---|---|
| Application of: | I hereby certify that this paper is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope addressed to: Commissioner of Patent and Trademarks, Washington, D.C. 20231, on this date: |
| Allen et al. | |
| Serial No: 06/933,553 | |
| Filed: 20 November 1986 | |
| For: ASSAY FOR SULFHYDRYL AMINO ACIDS AND METHODS FOR DETECTING AND DISTINGUISHING COBALAMIN AND FOLIC ACID DEFICIENCY | September 14, 1989 (Date) |
| Group Art Unit: 182 | /s/ Lewis S. Gruber Lewis S. Gruber (30,060) Attorney for Applicant(s) |
| Examiner: T. SCHEINER | |

AMENDMENT UNDER 37 C.F.R. §1.115

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sirs:

In an Office Action mailed May 26, 1989 (the period for response to which is extended to September 26, 1989 by a Petition for Extension of Time enclosed herewith) claims 13 and 15-17 were rejected under 35 U.S.C. §102(a) or (b) and claims 29-32 were rejected under 35 U.S.C. §103. Claims 1-12, 14 and 18-28 were indicated to be free of the prior art of

record. In response, reconsideration is respectfully requested in light of the following amendments and remarks.

In the Claims

Please amend claim 13 and please add claims 33 and 34 as follows.

13. (Amended) A method for detecting a deficiency of cobalamin or folate in warm-blooded animals [by] comprising the steps of:

assaying a body fluid[s] for [the presence of] an elevated level[s] of total homocysteine[.]; and

correlating an elevated level of total homocysteine in said body fluid with a deficiency of cobalamin or folate.

33. The method for detecting a deficiency of cobalamin or folate, as recited in claim 13, wherein said assaying *comprises the* step of determining an elevated level of total homocysteine in a human body fluid.

34. The method for detecting a deficiency of cobalamin, folate, or both, as recited in claim 18, wherein said assaying step comprises the step of determining elevated levels of total homocysteine and methylmalonic acid in a human body fluid.

REMARKS

Claims 1-32 are pending to the present application. In the above-cited Office Action, claims 1-12, 14 and 18 were noted by the Examiner to be tree of the prior art of record.

Saetre et al., Anal. Biochem., 90, 684-697 (1978) and Refsum et al., Clin. Chem., 31(4) 624-628 (1985), cited on page 12 of the Specification, were indicated to be unavailable to the Examiner. It was requested that the Applicants supply these references.

In response, copies of Saetre et al. and Refsum et al. are enclosed.

The Examiner noted that references supplied by Applicants in their Information Disclosure Statement of April 20, 1989 had been considered, however, a listing of the references on PTO-Form .1449 to complete the record would be greatly appreciated.

In response, a PTO-Form 1449 listing the previously-cited references is enclosed.

It should be noted that the designations of Mudd et al., Refsum et al. and Saetre et al as Documents 4, 7 and 9 in the enclosed PTO-Form 1449 are those of the previously submitted Information Disclosure Statement and not those in the PTO-Form 892 b[e] mailed by the Examiner with the Official Action on May 26, 1989.

A Supplemental Information Disclosure Statement and PTO-Form 1449 addressing additional art are also enclosed.

Since the filing of the last response, it was noted that the group of claims related to claim 13 and the group of claims related to claim 18 had no member specifically reciting a step for use with a human subject. Inasmuch as the Examples are directed to and support practice of the present invention with human subjects, it was believed to be appropriate to amend the application to add claims specifically directed to human uses.

Because new claims 33 and 34 properly depend from claim 13 and claim 18, respectively, and because each is a proper species of the genus of the claim from which it depends, it is submitted that no new search is required, and that claims 33 and 34 should be entered as proposed. All claims of the present application are believed to be in condition for allowance.

A. The Rejections under 35 U.S.C. §102 (a) or (b) May Properly Be [W]ithdrawn

In the above-cited Office Action claims 13 and 15-17 were rejected under 35 U.S.C. §102(a) or (b) as being anticipated by Applicants' admitted state of the art.

In the absence of a correlation step, the preamble of claim 13 was asserted by the Examiner to merely recite an intended use of the invention in that the claim lacked a positive limitation for correlating to a particular condition and had only one method step recited. The Examiner further asserted that Applicants admitted on pages 12 and 13 of the specification that assays for homocysteine were known, and that, with respect to claims 15-17, Applicants admitted that measurement of homocysteine by HPLC was conventional [citing Saetre et al., Anal. Biochem., 90, 684-697 (1978)], as was measurement of homocysteine converted to S-adenosylhomocysteine [citing Refsum et al., Clin. Chem., 31(4) 624-628 (1985)].

In response claim 13, and hence claims 15-17 by dependency therefrom, has been amended to recite a second step of correlating an elevated level of total homocysteine with a deficiency of cobalamin or folate.

This amendment was made without any intention to abandon any subject matter within the scope of the claims as originally filed and it is intended that claims of .the scope of the claims as originally tiled may be pursued in continuation or continuation-in-part applications.

Claims 13 and 15-17 go beyond the mere recitation of an intended use to provide a two step method which includes a correlating step not found in Refsum et al., or Saetre et al. Specifically, neither Refsum et al. nor Saetre et al. correlate levels of homocysteine in a body fluid with cobalamin or folate deficiency.

Inasmuch as a proper reference under 35 U.S.C. §102(a) or (b) must disclose all elements of a claim and inasmuch as Refsum et al. and Saetre et al. do not recite the step correlating homocysteine levels to a cobalamin or folate deficiency, Refsum et al. and Saetre et al. are not proper references against claims 13 and 15-17 under 35 U.S.C. §102(a) or (b) and the rejection under this section should be withdrawn.

B. The Rejection under 35 U.S.C. §103 May Properly Be Withdrawn

In the above-cited Office Action claims 29-32 were rejected under 35 U.S.C. §103 as being unpatentable over Applicants' admitted state of the art upon the Examiner's assertion that, on page 3 of the Specification, and at numerous other locations, Applicants admitted that treatment of diagnosed cobalamin or folic acid deficiencies by administration of cobalamin or folic acid, respectively, was conventional in the art. The Examiner further asserted that it would have been obvious for one of ordinary skill in the art to relieve the symptoms of cobalamin or folate deficiency, regardless of the method of diagnosis, by administering cobalamin or folic acid. Incorporation of the instant method of diagnosis into an old treatment protocol was asserted not to provide unexpected results so that this could not be considered unobvious [citing In re Durden, 226 USPQ 395].

In response, it is respectfully submitted that In re Durden relates to the use of a new starting material in an otherwise old process or to the production of a new product in a process which is otherwise old. See In re Durden, 226 USPQ 359, 362 (Fed. Cir. 1985). In claims 29-32 of the present application, a new combination of process steps its provided, a situation to which In re Durden is not directed.

Moreover, the Examiner's comment that it is obvious to relieve symptoms of cobalamin or folate deficiency by administering cobalamin or folate is the basis for an improper rejection of a method claim on the basis that some of its steps are old. In fact, the court in In re Durden did address the issue of whether a claim must be considered as a whole, and indicated that it did.

The Applicant has not admitted, and the Examiner has not asserted, that there was in the prior art a suggestion to combine the steps recited in claims 29-32. Absent a proper suggestion in cited art to combine the steps, the combination of steps of claims 29-32 is not properly rejected as obvious.

Furthermore, the diagnostic steps of processes which are admitted by the Examiner to be free of the art of record (i.e.,

the steps of claim 18 referenced in claims 30 and 32) or which are established in Section A of this Amendment to be free of art (i.e., the steps of claim 13 referenced in claims 29 and 31) are novel and nonobvious and render novel and nonobvious all claims in which they are recited in combination with other elements. In this regard, it is respectfully submitted that it is improper to reject claims 29-32 as obvious over art in combination with the Applicants' invention of claims 13 and 18.

Therefore, because the method claimed in claims 29-32 is new and nonobvious, unlike the process of In re Durden which process was old but was sought to be patented on the basis of its application to a new starting material, the rejection under 35 U.S.C. §103 should be withdrawn.

Summary

In light of the foregoing amendments and remarks, it is believed that the rejections for obviousness and under 35 U.S.C. §102(a) or (b) and under 35 U.S.C. §103 have been overcome and that claims 13, 15-17 and 29-32, along with all other claims in the present application, are now in condition for allowance.

Respectfully submitted,

MARSHALL, O'TOOLE,
GERSTEIN,MURRAY
& BICKNELL

BY: /s/ Lewis S. Gruber

Lewis S. Gruber (Reg. No. 30,060)
Two First National Plaza Suite 2100
Chicago, IL 60603
(312) 346-5750

**Defendant's Exhibit T**

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|---|---|---|
| Application of: | ) | I hereby certify that this |
| | ) | paper is being deposited |
| Robert H. Allen et al. | ) | with the United States |
| | ) | Postal Service as first |
| Serial No: 06/933,553 | ) | class mail, postage prepaid, |
| | ) | in an envelope addressed to: |
| Filed: 20 November 1986 | ) | Commissioner of Patents and |
| | ) | Trademarks, Washington, D.C. |
| For: Assay for Sulfhydryl | ) | 20231, on this date: |
| Amino Acids and Methods for | ) | |
| Detecting and Distinguishing | ) | September 14, 1989 |
| Cobalamin and Folic Acid | ) | (Date) |
| Deficiency | ) | |
| | ) | /s/ Lewis S. Gruber |
| Group Art Unit: 182 | ) | Lewis S. Gruber (30,060) |
| | ) | Attorney for Applicant(s) |
| Examiner: T. Scheiner | ) | |

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. §1.97 INCLUDING FORM PTO-1449

TO: Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

Sirs:

In compliance with Title 37 of the Code of Federal Regulations, Sections 1.97, et seq., enclosed is a Form PTO-1449 listing documents disclosed to the Examiner by this Information Disclosure Statement.

The significance of each document cited on the Form PTO-1449 is discussed below. However, if further comment on any reference would be of assistance to the Examiner, advice of same is solicited. In addition, the Examiner is urged to

undertake an independent review of the documents to assure that all aspects of the documents relevant to the present invention are considered.

Document C18, Stabler et al., Blood, 66 (Suppl.), Abstract 87, 50a (Am. Soc. Hem. Meeting) (1985) is a publication coauthored by the Applicants and published less than one year prior to the filing date of the present application. Document C18 relates to an assay for serum homocysteine which is stated to be converted to methionine by the cobalamin-dependent enzyme methionine synthetase which also converts homocysteine and methyltetrahydrofolate. Also in Document C18, it is stated that infusion of a cobalamin analogue, OH-cobalamin(c-lactam], reduced mean rat liver methionine synthetase activity and increased mean serum homocysteine. Exposure to 50% nitrous oxide for 36 hours is reported to have reduced mean synthetase and increased mean serum homocysteine. Injections of OH-cobalamin are reported to have elevated mean synthetase and decreased mean serum homocysteine.

In Document C18, it is concluded that: 1) homocysteine can be detected and quantitated in normal serum, 2) serum homocysteine is elevated in almost all untreated patients with cobalamin or folate deficiency and will be useful diagnostically, and 3) serum homocysteine is a sensitive indicator of methionine synthetase activity in humans and rats.

In a telephone conference with Michael de Nardo, a person represented by the publisher of Blood as having records of distribution data for issues of this journal in November 1985, Mr. de Nardo reported that the mailing date for the Volume 66 Abstract issue (November 1985) of Blood was November 24, 1985. As a publication less then one year prior to the date of the present application and deriving its disclosure from the Applicants, Document C18 is not a proper reference against the present application.

Moreover, Document C18 does not disclose the combination of assays for MMa and homocysteine which may be used to distinguish cobalamin and folate deficiencies and to treat such deficiencies according to the present invention.

Document C3, Brattstrom et al., Metabolism, 34(11), 1073-1077 (1985), relates to homocysteinemia and the observation that administration of folic acid to individuals whose folates and cobalamins are above or within normal ranges reduces homocysteine. Document C3 is concerned with reducing circulating homocysteine concentration in nonfolate-deficient men and women (see page 1076).

No suggestion is made that folate or cobalamin deficiency is related to homocysteinemia. In this regard excess folate is described, at page 1076, as a mass action effector to cause conversion of excessive homocysteine to methionine not as therapy for a deficiency.

Although it is noted that deficiencies of certain enzymes in pathways involving homocysteine or methionine result in homocysteinemia, Document C3 does not disclose the particular problems of assaying for sulfhydryl amino acids disclosed in the present application, nor does Document C3 suggest the solution to the problems according to the present invention as recited in claim 1, specifically, mixing a labeled reference standard with a sample, adding sufficient reducing agent to randomize the labeled and unlabeled sulfhydryl amino acids, and measuring the amount of labeled and unlabeled sulfhydryl amino acid present. Moreover, Document C3 does not disclose any advantage of an assay for MMa or of an assay for a sulfhydryl amino acid, such as homocysteine, or both with respect to the diagnosis or treatment of cobalamin or folate deficiency.

Thus, although Document C3 relates to treatment of homocysteinemia with folic acid, it does not disclose or suggest proper combination with other documents of record to disclose the method of assaying for a sulfhydryl amino acid or the use of assays for MMa or homocysteine or both for diagnosis and treatment of cobalamin or folate as claimed in the present application.

* * * *

**Defendant's Exhibit Z**

University Science, Engineering and Technology, Inc.
1465 Post Road East
P.O. Box 915
Westport, CT 06881-0915
(203) 259-7997

October 26, 1990

**CONFIDENTIAL**

Dr. Robert H. Allen
President
Metabolite Laboratories, Inc.
4001 S. Dexter Street
Inglewood, Colorado 80110

Re: CU 280/281 - Assay for Sulfhydryl Amino Acids and Methods for Detecting and Distinguishing Cobalamin and Folic Acid Deficiency
License Agreement: UPI and Metabolite Laboratories, Inc.
Dated as of 22 November, 1988 (the "License Agreement")

Dear Dr. Allen:

You have advised me that Metabolite Laboratories, Inc. ("Metabolite") desires to enter into a relationship with Roche Diagnostics (and perhaps other entities as well) under which Metabolite will sublicense the rights granted to it by UPI in the License Agreement. Further, you have advised me that the ability of Metabolite to sublicense may enhance the rate of commercial exploitation of the invention. In consideration of the foregoing, University Patents, Inc., as licensor under

the License Agreement, agrees to amend, and does hereby amend the License Agreement, as follows:

1. Section 2.02 of the License Agreement is deleted in its entirety, and in substitution thereof, is inserted:

"Section 2.02 During the term of this Agreement, the license hereby granted shall include the right of LICENSEE to grant written sublicenses, provided that LICENSEE shall include all Sales made by all sublicensees in its reports to UPI as provided in Section 4.03 and pay royalties to UPI as though all such transactions by sublicensees were in fact made by LICENSEE hereunder. LICENSEE agrees to deliver to UPI a notice of each and every sublicense entered into by LICENSEE within thirty (30) days after execution thereof and shall promptly advise UPI in writing of any modification or termination of each sublicense. Each notice shall contain the name and address of each sublicensee, the title or designation of its responsible officer, the term and geographic limitations, if any, of the sublicense, and shall contain a representation that the sublicensee has been made aware of the essential terms of this Agreement, including (by way of example and not limitation) the following provisions: (1) royalty obligations; (2) accounting; (3) default; (4) warranty; (5) assignment; (6) indemnification and (7) restriction on the use of the name of the University of Colorado. Upon termination of this Agreement for any cause, any and all existing sublicenses hereunder shall thereupon be assigned to UPI. This shall be made a condition of any such sublicense that may be granted by LICENSEE."

2. In all other respects, the License Agreement is hereby affirmed and ratified and remains in full force and effect.

Sincerely,

University Patents, Inc.

By: /s/ [Illegible]
Vice President

ACCEPTED AND AGREED TO
This 2nd day of November, 1990

Metabolite Laboratories, Inc.

By: /s/ Robert H. Allen
President

RIS/mbe

cc: J. Holloway
H. Jacobson
J. Lindenbaum

**Defendant's Exhibit AB**

University of Colorado Health Sciences Center
Department of Medicine
Division of Hematology
Campus Box B170 School of Medicine
4200 East Ninth Avenue
Denver, Colorado 80262
(303) 270-8474

June 4, 1992

Dr. Peter W. Wentz
Director of Toxicology
Roche Biomedical Laboratories
1447 York Court
Burlington, NC 27296

Dear Peter,

As we discussed on the telephone yesterday, I am very pleased that things are running smoothly with you in terms of the performance of the metabolite assays. I will always be glad to answer any technical questions that arise concerning the metabolite assays and Dr. Sally P. Stabler and myself will be glad to answer any questions that physicians might have regarding the results or interpretations of the metabolite assays.

One aspect of the metabolite assays that I did not mention in our conversations concerns the occasional request that only one of the metabolites be performed rather than all four. I have always refused to do this because I do not believe that it is good medical practice. My reasoning is based on the fact that a normal value for one metabolite does not exclude the possibility that one or more of the additional metabolites may be elevated as well as the fact that an isolated elevated value for one metabolite can not be interpreted completely without knowing whether the other metabolites are also

elevated or are normal. There are also technical and possible economic reasons for not separating the metabolite assays but these are overshadowed by the importance of good medical practice as just noted above. If you have any questions about this aspect of the metabolites or if this becomes an issue in the future, I hope you will feel free to ask me for any assistance that I might provide in this matter.

I am also enclosing four tables that might serve as a starting point for a brochure or physician education piece about the indications, utility and interpretation of the metabolite assays. You may want to alter the normal ranges that I have listed for serum vitamin $B_{12}$ and folate to match those of Roche Biomedical Laboratories and I hope that you and your marketing people will feel free to make any other changes that you believe would be helpful. I am also enclosing reprints of several articles that you may wish to list as a bibliography at the end of an education piece regarding the metabolite assays.

Please let me know if I can be of any further assistance.

With best regards,

Sincerely yours,

/s/ Bob
Robert H. Allen, M.D.
Professor of Medicine
Professor of Biochemistry
Director, Division of Hematology

RHA:vla

Enclosures

**Defendant's Exhibit AC**

AGREEMENT

This Agreement effective as of the 31st day of January, 1991 ("Effective Date") by and between Hoffmann-La Roche Inc., a New Jersey corporation having offices at 340 Kingsland Street, Nutley, New Jersey 07110 (hereafter "HLR") and Metabolite Laboratories, Inc., a Colorado corporation, having offices at No. 4001 S. Dexter Street, Englewood, Colorado 80110 (hereafter "MLI");

WITNESSETH

WHEREAS, MLI represents and warrants that it has the full power and authority to grant sublicenses under the invention disclosed and claimed in Un[it]ed States Patent 4,940,658, issued July 10, 1990, entitled "Assay for Sulfhydryl Amino Acids and Methods for Detecting and Distinguishing Cobalamin and Folic Acid Deficiency", inventors Robert H. Allen, Sally B. Stabler and John Lindenbaum including any patents issuing from any continuations, continuations-in-part, divisions and/or reissues thereof (hereafter "Licensed Patents") as evidenced in part by a License Agreement between University Patents, Inc. (hereafter "UPI") and MLI dated November 22, 1988, and a letter amendment thereof dated October 26, 1990 granting MLI the right to sublicense (hereafter collectively referred to as "Prior Agreement"), a copy of which are attached to this Agreement and have been reviewed by HLR;

WHEREAS HLR is a major healthcare company which, through its Roche Biomedical Laboratories (hereafter "RBL") subsidiary, provides commercial clinical laboratory services to the medical community and is desirous to obtain rights to commercially offer (i) assays of homocysteine using methods and materials falling within the claims of the Licensed Patents; (ii) assays of methylmalonic acid utilizing

mass spectrometry or using methods and materials falling within the claims of the Licensed Patents; (iii) assays of 2-methylcitric acid utilizing mass spectrometry or used to detect Vitamin $B_{12}$ or Folic Acid deficiencies; and (iv) assays of cystathionine utilizing mass spectrometry or used to detect Vitamin $B_{12}$ or Folic Acid deficiencies (hereafter collectively referred to as the "Licensed Assays");

WHEREAS MLI is agreeable to extend a sublicense under Licensed Patent to HLR and to grant HLR certain rights to commercially utilize future improvements, whether or not patentable, to Licensed Assays and to proprietary technology relating to automated apparatus for carrying out Licensed Assays which may be developed by MLI during the term of this Agreement (hereafter collectively "Licensed Rights") in return for HLR's commitment to be bound by all relevant terms and conditions of Prior Agreements and its agreement to the undertakings set forth in this Agreement;

NOW, THEREFORE, in consideration of these premises and of the mutual covenants hereinafter set forth, HLR and MLI mutually covenant and agree as follows:

## ARTICLE I - GRANTS

1.01 Subject to the terms and conditions of this Agreement, MLI agrees to grant and hereby grants to HLR a nonexclusive license to the Licensed Rights and a nonexclusive sublicense under the Prior Agreements to the Licensed Patents which license and sublicense shall be for the practice of Licensed Assays in the United States.

1.02 MLI agrees that promptly after the Effective Date it will provide to RBL, at a place and in a manner reasonably designated by RBL, calibrators used in performance of the Licensed Assays and all technology and know-how including technology relating to automated apparatus reasonably needed by RBL to carry out commercial operations with Licensed Assays at no additional cost to RBL. RBL will be free

to apply the technology and know-how relating to the automation of the assay to any related or unrelated assay not covered by this Agreement without additional consideration.

1.03 MLI agrees to provide RBL, at RBL's request, with the right to refer samples to MLI and MLI will conduct Licensed Assays on such samples (hereafter "Referral Assays") and provide RBL with the results of such test in accordance with normal business practice.

ARTICLE II - PAYMENTS UNDER THE LICENSES

2.01 In consideration of the grants made in ARTICLE I, HLR agrees to pay to MLI a royalty of 43% in respect to HLR's Net Sales (as defined below) of Referral Assays.

2.02 In consideration of the grants made in ARTICLE 1, HLR agrees to pay to MLI a royalty of 21.5% in respect to HLR's Net Sales of Licensed Assays other than Referral Assays.

2.03 Additionally, HLR agrees to pay to UPI or an affiliated company designated by UPI, all royalties due to UPI under the terms and conditions of Prior Agreement on Sales (as defined in Prior Agreement) of Referral Assays and Licensed Assays.

2.04 The term "Net Sales" as used herein shall mean the sales price of Licensed Assays or Referral Assays actually received by HLR or its Affiliates (as defined herein) upon sales to first customers of such Assays after deducting returns, sales rebates, cash discounts, sales taxes or other taxes directly linked to the sale of such Assays. Sales of such Assays between or among HLR and its Affiliates shall not be considered in the computation of Net Sales, but sales by Affiliates of HLR to customers of HLR or its Affiliates

shall be included. For purposes of this Section 2.04, the term "Affiliate" shall mean:

i) a business entity which owns, directly or indirectly, a controlling interest in either party, by stock ownership or otherwise; or,

ii) a business entity which is owned by either party, either directly or indirectly, by stock ownership or otherwise; or,

iii) a business entity, the majority ownership of which is directly or indirectly common to the majority ownership of either party;

iv) Affiliate shall not mean Genentech, Inc.

## ARTICLE III - ACCOUNTING

3.01 Royalty payments due hereunder in connection with Net Sales of Licensed Assays other than Referral Assays shall be computed on a quarterly accounting period ending on the last day of each calendar quarter for each License Year (as defined in Prior Agreement) during the term of this Agreement. Payments hereunder shall be made within sixty (60) days following each quarterly period, and each payment shall include the royalties during the most recent quarterly accounting period. Royalty payments in connection with Net Sales of Referral Assays will be invoiced by MLI on a monthly basis with payment due within thirty (30) days of receipt of invoice by HLR. Payments to UPI pursuant to Section 2.03 shall be made in the manner and at the times set forth in Article IV of the Prior Agreements.

3.02 Payments made hereunder shall be made in United States Dollars.

3.03 All royalty payments made in accordance with 3.01 shall be accompanied by a report certified by an officer of HLR setting forth the quantity of Licensed As-

says conducted by or on behalf of HLR and its Affiliates and documentation of the basis for calculating the Net Sales and Sales made by HLR and its Affiliates to include copies of any reports wherefrom Net Sales and Sales can be determined and the calculation of the royalties payable can be made. Such reports shall also be made if no royalties are due. For a period of two (2) years after each royalty period, HLR shall keep records pertaining to such royalty period in sufficient detail to permit the determination of royalties payable hereunder and at the request and expense of MLI will permit an independent Certified Public Accountant, selected by MLI and acceptable to HLR, or any other person acceptable to both MLI and HLR, to examine during ordinary business hours once in each calendar year such records as may be necessary to verify or determine royalties paid or payable under this Agreement. Such Certified Public Accountant or other person shall report to MLI only the amount of royalties due and payable.

3.04 Upon termination or expiration of this Agreement, HLR shall pay to MLI and UPI within sixty (60) [d]ays after such termination or expiration, all unpaid royalties accrued until such time.

## ARTICLE IV - TERMINATION

4.01 The term of this Agreement shall be from the Effective Date until the date of expiration of the last to expire of the Licensed Patents.

4.02 HLR shall have the right at any time on sixty (60) days prior written notice to MLI to terminate this Agreement with respect to a particular Licensed Assay if (i) in the case of Licensed Assays of homocysteine, a more cost effective commercial alternative is available that does not infringe a valid and enforceable claim of the Licensed Patents; (ii) in the case of Licensed Assays of methylmalonic acid, a more cost

effective commercial alternative is available that does not utilize mass spectrometry and does not infringe a valid and enforceable claim of the Licensed Patents; (iii) in the case of Licensed Assays of 2-methylcitric acid, a more cost effective commercial alternative is available that does not utilize mass spectrometry and is not used to detect Vitamin $B_{12}$ or Folic Acid deficiencies; and (iv) in the case of Licensed Assays of cystathionine, a more cost effective commercial alternative is available that does not utilize mass spectrometry and is not used to detect Vitamin $B_{12}$ or Folic Acid deficiencies. MLI shall have the right on sixty (60) days prior written notice to HLR to terminate this agreement if there has been no payment made to MLI during any eight (8) consecutive quarters if HLR permanently discontinues offering Licensed Assays and Referral Assays.

4.03 MLI shall have the right to terminate this Agreement on sixty (60) days prior written notice in the event (i) that HLR is in material breach of a provision of this Agreement and such breach is not cured during such notice period then MLI can terminate in thirty (30) days on providing a second written notice; or (ii) that HLR shall become bankrupt or insolvent, or if HLR's business shall be placed in the hands of a receiver, assignee or trustee whether by the voluntary act of HLR in the course of in[s]olvency proceedings. MLI shall have the right to discontinue conduct of Referral Assays during the continuance of any material breach of a provision of this Agreement by HLR if such breach shall be uncorrected twenty (20) days after the written notice of such breach.

4.04 Any termination under this ARTICLE IV shall not relieve HLR or MLI of any obligation or liability accrued hereunder prior to termination, or rescind or give rise to any right to rescind anything done or any payments made or other considerations given here-

under prior to the time such termination becomes effective; and such termination shall not affect in any manner any remedy of either party arising out of this Agreement prior to such termination.

4.05 Upon termination of the Prior Agreements between UPI and MLI for any reason, the sublicense under the Prior Agreements to the Licensed Patents granted to HLR hereunder shall continue in full force and effect and shall be deemed to be assigned by MLI to UPI. It is expressly understood that such assignment shall not operate to expose HLR to any further obligations than are set forth in this Agreement.

## ARTICLE V - NOTICE

5.01 Any payment, notice or other communication required or permitted to be made or given to either party hereto pursuant to this Agreement shall be sufficiently made or given on the date of mailing if sent to such party by certified or registered mail, postage prepaid, addressed to it at its address set forth below, or to such other address as it shall designate by written notice given to the other party as follows:

In the case of MLI:

President
Metabolite Laboratories, Inc.
4001 S. Dexter Street
Englewood, Colorado 80110

In the Case of HLR:
Notices to be sent to:

Roche Biomedical Laboratories
430 Spring Street
Burlington, North Carolina 27215
Atten: Brad Smith, Esq. and Mr. Ernie Knesel

With copy to:

Corporate Secretary
Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, New Jersey 07110

5.02 MLI agrees that it will be responsible for all notices regarding the extension of a sublicense under Prior Agreement which may be required to be made to UPI and, except as provided in Section 2.03, shall take all steps needed to maintain the Prior Agreement in full force and effect during the term of this Agreement.

## ARTICLE VI - MISCELLANEOUS PROVISIONS

6.01 MLI warrants and represents that it has the full right and power to enter into this Agreement, and that there are no outstanding agreements, assignments, or encumbrances inconsistent with the provisions of this Agreement other than as may be expressly set forth herein. MLI represents that it is aware as of the Effective Date of no facts that would render Licensed Patents invalid or unenforceable. MLI makes no other representation or warranty, express or implied, including warranty, guaranty or representation as to the safety and efficacy of the Licensed Assays, nor does MLI assume any liability in respect of any infringement of patents or other rights of third parties due to HLR's operation under the license and sublicense herein granted. MLI represents that it is not aware as of the Effective Date of any claim or assertion by any third party that the performance of Licensed Assays or Referral Assays would infringe patent rights or other rights of such third parties.

6.02 All services and other transactions between the parties with respect to commercial activities in support of Referral Assays and/or Licensed Assays shall be made in accordance with the respective party's then established customary business terms generally of-

fered to the trade. In the event of a conflict between such customary business terms and the express terms of this Agreement then the express terms of this Agreement shall control.

6.03 This Agreement and the license rights granted shall not be assignable by HLR without the prior written consent of MLI, which shall not be unreasonably withheld, except that HLR may assign this Agreement and the license rights herein granted in connection with the sale and transfer of substantially all of the business assets of RBL, or of HLR's consolidation or merger with another company; provided that such assignee shall assume, in writing, all of the obligations of HLR hereunder.

6.04 Each party agrees that nothing contained herein shall be construed as conferring any right to use in advertising, publicity or other promotional activities in the name, insignia, symbols or other designation of the other party to this Agreement or any other party of the Prior Agreement with respect to any promotional activity associated with this Agreement or assays licensed hereunder without the written consent of the other party.

6.05 This Agreement shall be construed and the legal relations between the parties determined, in accordance with the laws of the State of New Jersey.

6.06 If any provision of this Agreement shall be held to be invalid, illegal, or unenforceable, the validity, legality and enforceability of the remaining provisions shall not in any way be affected or impaired thereby.

6.07 The headings of the several sections are inserted for convenience of reference only and are not intended to be part of or to affect the meaning or interpretation of this Agreement.

6.08 This Agreement embodies the entire understandings of the parties and supercedes any other agreement or understandings between the parties relating to the subject matter hereof. No waiver, amendment or modification of this Agreement shall be valid or binding upon such parties unless made in writing and signed on behalf of each such party by its respective proper officers thereto duly authorized.

6.09 All obligations and benefits made or received by HLR hereunder may be performed or received by RBL without prior notice to MLI.

## ARTICLE VII - MOST FAVORED LICENSEE

7.01 In the event that MLI shall grant any other party rights to Licensed Assays or Referral Assays at terms and conditions more favorable to such other party than are granted to HLR hereunder, then MLI agrees to give HLR prompt written notice of that fact and HLR shall have the right to receive such more favorable terms provided that HLR shall agree to be bound by all other relevant terms and conditions granted such other party.

## ARTICLE VIII - CONFIDENTIALITY

8.01 For the purposes of this Agreement, "Confidential Information" shall mean any information, whether written or oral, that is furnished or disclosed by MLI, or its employees, representatives or agents, relating to the Prior Agreements or the Licensed Assays or to the business, financial condition, strategies, plans, policies, clients or customers, inventions, trade secrets, computer programs, or processes of MLI. For the purposes of this Agreement, "Recipients" shall mean HLR and its Affiliates and their respective directors, officers, partners, employees, agents or representatives, and any or all of them, to the extent such persons receive Confidential Information.

8.02 (a) HLR agrees not to use the Confidential Information received from MLI for any other purpose except the purposes of this Agreement;

(b) to treat the Confidential Information received from MLI as it would its own proprietary information and to provide the Confidential Information to Recipients solely on a need to know basis; and,

(c) to take all reasonable precautions to prevent the disclosure of the Confidential Information received from MLI hereunder to any third party, other than Affiliates, without the express written consent of MLI. Upon termination, at MLI's request, HLR agrees to return all copies of Confidential Information to MLI, except HLR may retain one copy in a limited access file for archival purposes only.

IN WITNESS WHEREOF, the parties hereto have caused this Agreement to be duly executed as of the date first written above.

HOFFMANN-LA ROCHE INC.

By: /s/ Illegible

Title: Vice President

Date: 2-14-91

METABOLITE LABORATORIES INC.

By: /s/ Robert H. Allen

Title: President

Date: 2/6/91

**Defendant's Exhibit AH**

**COMPETITIVE TECHNOLOGIES**
1960 Bronson Road
Fairfield, CT 06430
(203) 255-6044

**Releases**

## American Heart Association Recommends Homocysteine Testing

Available Now, Simple Test Uncovers Potential Heart Problems - Likely to Become as Common as Cholesterol Testing

**FAIRFIELD, CT. (January 5, 1999)**—In the current issue of its weekly journal, "Circulation," an American Heart Association Science Advisory urges doctors to begin screening high-risk patents with a personal or family history of heart disease for elevated homocysteine levels.

The advisory comes in the wake of a number of recent studies, including a 14-year study at the Harvard School of Public Health involving 80,000 women, which concluded that high homocysteine levels play a central role in cardiovascular disease. An estimated 20 million Americans, more than one of every three people with heart disease, experience high levels of homocysteine. This means that screening for the problem could significantly reduce the likelihood of heart attacks and strokes among certain high-risk patients.

["]It will probably soon be as common to have one's homocysteine level checked as it is now to have one's cholesterol level checked," said Dr. Malinow of the Oregon Health Sciences University in Portland, a joint author of the Science Advisory.

Competitive Technologies, Inc. (Amex: CTT - news) supported and patented the technology now licensed to laborato-

ries performing in excess of 75% of homocysteine tests in the United States.

"We first became involved with this technology in the mid-1980s when we began working with the team from the University of Colorado and Columbia University that made important discoveries in the homocysteine area. We're now very happy to see it come to fruition," said Competitive Technologies' President Frank McPike. "We have always known that it takes many years to bring important medical technologies into the market, but we think the investment is worthwhile, especially when it delivers something that can actually save lives like this test. We plan to continue to work long and hard so we can keep bringing other equally useful and important technologies to the market.

Cardiovascular disease is the leading cause of death in the United States, killing one American every 33 seconds, a cost to the American economy of $150 billion a year. A test for high homocysteine blood levels may help reduce that number because elevated levels of homocysteine can be reduced with a relatively simple change in diet. Because high levels of homocysteine directly correlate to low levels of folic acid and vitamins B12 and B6, in addition to screening, the Science Advisory also urges all Americans to increase their intake of foods containing folic acid and vitamins B12 and B6 such as leafy green vegetables, fruits, and legumes.

At normal levels, homocysteine is an amino acid used by the body to help build and maintain body tissue. Too much homocysteine, however, causes blood platelets to clump together and damage the lining of arteries. That, in turn, can lead to heart attacks and strokes.

Because of the increasing importance associated with identifying homocysteine levels, the number of homocysteine tests performed could top 100 million per year. CTT's share of retained royalties from its homocysteine licensees during the first fiscal quarter of 1999 has already increased over 200% from the corresponding period of 1998. The average price of a test is $53. In addition to cardiovascular disease, high levels of homocysteine have also been linked to Alzheimer's

disease, Chronic Fatigue Syndrome and Rheumatoid Arthritis. CTT's U.S. Patent No. 4,940,658 expires in 2007.

Competitive Technologies is a global leader in the identification, development and commercialization of innovative technologies in the areas of life sciences, physical sciences and digital technologies. Competitive Technologies' specialized expertise and experience make it a valuable partner for companies of all sizes within the area of technology commercialization. CTT has evaluated more than 25,000 technologies and has been responsible for closing hundreds of license agreements.

Important CTT clients and licensees include: SONY (NYSE: SNE - news), Lucent Technologies (NYSE: LU), Matsushita Electric Industrial (NYSE: MC), and Ribozyme Pharmaceuticals, Inc. (Nasdaq: RZYM) among others. Competitive Technologies, Inc. is based in Fairfield, CT with affiliates in Osaka, Japan and London, England.

Statements about the Company's future expectations included development and regulatory plans, and all other statements in this document other than historical facts are "forward-looking statements" with the meaning of applicable Federal Securities laws and are not guarantees of future performance. These statements involved risks and uncertainties related to the market acceptance of and competition for the Company's licensed technologies and other risks and uncertainties inherent in CTT's business. These are set forth in Item 1 of the Company's Form 10-K for the year ended July 31, 1998. Other factors may also be described in CTT's filings with the SEC and are subject to change at any time. The Company's actual results could differ materially from these forward-looking statements.

**Defendant's Exhibit AI**

**COMPETITIVE TECHNOLOGIES**
1960 Bronson Road
Fairfield, CT 06430
(203) 255-6044

**Releases**

**CTT's Homocysteine Technology Hits Main Street**

- American Heart Association Alerts Doctors to Screen High-Risk Patients

- Available Today, Homocysteine Test May Become as Common as Cholesterol Test

**FAIRFIELD, CT. (January 5, 1999)**—After a year of promoting its homocysteine technology, **Competitive Technologies, Inc. (Amex: CTT)** is seeing its hard work pay off. Competitive Technologies, Inc., a global leader in technology commercialization, holds the patent which covers the performance of any test used to determine elevated homocysteine levels. The number of those tests may now be about to rise dramatically.

Scores of medical studies reveal that high levels of homocysteine may indicate a greater risk of vascular disease. In the wake of those studies, the American Heart Association (AHA) has released a Science Advisory urging doctors to immediately begin screening high-risk patients with a personal or family history of heart disease. The advisory will be featured in the upcoming January 5-12, 1999, issue of Circulation: Journal of the American Heart Association.

Though the number of homocysteine tests has been rising rapidly over the past year, they may increase even more quickly now. "It will probably soon be as common to have one's homocysteine level checked as it is now to have one's cholesterol level checked," said Dr. Malinow of the Oregon

Health Sciences University, a joint author in the AHA Science Advisory.

If that prediction proves true, the number of homocysteine tests could approach the level of cholesterol tests which has been estimated between 100 and 125 million tests per year in the United States alone. An estimated 20 million Americans, more than one of every three people with cardiovascular disease, have high levels of homocysteine.

"The American Heart Association's recommendation is important news," said CTT President Frank McPike. "Heart attacks kill more Americans than any other disease, and this recommendation certainly confirms the importance of homocysteine testing as a tool in the battle to reduce heart disease. This test is available today and we now expect physicians to increasingly encourage their patients who are at risk for heart disease to regularly monitor their homocysteine levels."

CTT believes it is now positioned to collect royalties on at least 75% of all homocysteine tests performed in 1999. According to McPike, CTT's retained royalties from its homocysteine licensees during the first quarter of fiscal 1999 has already increased more than 200% over the corresponding period in 1998, with the average price of a test being approximately $53. In addition to collecting royalties on more tests in 1999, CTT expects to increase its market share by targeting smaller diagnostic laboratories during the next six months.

CTT, which identifies and invests in incubator-stage technologies in return for licensing fees, royalties or equity, first became involved in homocysteine during the mid-1980's. The technology resulted from research performed at the University of Colorado by Drs. Robert H. Allen, Sally P. Stabler, and the late Dr. John Lindenbaum of Columbia University. In addition to cardiovascular disease, high levels of homocysteine have been linked to Alzheimer's disease, Chronic Fatigue Syndrome and Rheumatoid Arthritis. CTT's U.S. Patent No. 4,940,658 expires in 2007.

Competitive Technologies is a global leader in the identification, development and commercialization of innovative technologies in the areas of life sciences, physical sciences and digital technologies. Competitive Technologies' specialized expertise and experience make it a valuable partner for companies of all sizes within the area of technology commercialization. CTT has evaluated more than 25,000 technologies and has been responsible for closing hundreds of license agreements.

Important CTT clients and licensees include: SONY (NYSE: SNE), Lucent Technologies (NYSE: LU), Matsushita Electric Industrial (NYSE: MC), and Ribozyme Pharmaceuticals, Inc. (Nasdaq: RZYM) among others. Competitive Technologies, Inc. is based in Fairfield, CT with affiliates in Osaka, Japan and London, England.

Statements about the Company's future expectations included development and regulatory plans, and all other statements in this document other than historical facts are "forward-looking statements" with the meaning of applicable Federal Securities laws and are not guarantees of future performance. These statements involved risks and uncertainties related to the market acceptance of and competition for the Company's licensed technologies and other risks and uncertainties inherent in CTT's business. These are set forth in Item I of the Company's Form 10-K for the year ended July 31, 1998. Other factors may also be described in CTT's flings with the SEC, and are subject to change at any time. The Company's actual results could differ materially from these forward-looking statements.

**Defendant's Exhibit AM**

The Lancet, December 27, 1969

## HOMOCYSTINURIA AND ORGANIC ACIDURIA IN A PATIENT WITH VITAMIN-$B_{12}$ DEFICIENCY

SIR,—The letter of Dr. Shipman and his colleagues (Sept. 27, p. 693) suggesting genetic linkage of homocystinuria and the intrinsic-factor-deficiency type of pernicious anemia to a G chromosome prompts us to report our finding of homocysteine in the urine of a patient with Imerslund's syndrome – i.e., familial pernicious anemia and proteinuria. (In this syndrome the malabsorption of vitamin $B_{12}$ occurs in the presence of intrinsic factor.)

Our patient, a 10-year-old White boy, was taking oral vitamins when he was referred to us because of nausea, vomiting, malaise, proteinuria, anemia, and ataxia. He was lethargic and pale. The glossal papillae were strophic, there were fissures at the angles of the mouth, and the liver was enlarged. He was ataxic, and position and vibration sense were impaired. Hemoglobin was 8·2 g. per 100 ml.; hematocrit 24%; red-blood-cell count 2,190,000 per c.mm.; reticulocytes 0·7%; platelet-count 237,500 per c.mm. The blood-film showed macrocytes (M.C.V. 100 c.mm., M.C.H. 37·9 µg., M.C.H.C. 35%) and hypersegmented neutrophils. Bone-marrow was megaloblastic. Serum glutamic-oxaloacetic and glutamic-pyruvic transaminases (S.G.O.T. and S.G.P.T.) and lactic-dehydrogenase (L.D.H.) were raised. Alkaline phosphatase and bilirubin were normal. Serum-carotene was 21·5 µg. per 100 ml.; serum-$B_{12}$ was less than 50 pg. per ml. Schilling test: 0·2% of orally administered vitamin $B_{12}$ was excreted in 24 hours, and with intrinsic factor 1·1% was excreted. Gastic analysis showed free acid which increased after betazole ('Histalog'). A gastric biopsy specimen and D-xylose absorption were normal. Aminoacid and organic-acid levels are shown in the accompanying table.

Two weeks after parenteral vitamin $B_{12}$ treatment (total 3 mg.), the hemoglobin rose, and neurological function improved. The liver was no longer enlarged. At this time, urinary methylmalonic acid, fumaric acid, and homocystine were not measurable by the methods used.

The vitamin-$B_{12}$ malabsorption in this patient appears to have a different genetic basis from that in the case reported by Dr. Shipman and his colleagues, yet homocystine was present in the urine in significant amounts. We interpret the homocystinuria in our case, like the methylmalonic aciduria, as being secondary to vitamin-$B_{12}$ deficiency, an effect found in the vitamin $B_{12}$ metabolic error reported by Mudd et al. This view was strengthened when urinary homocystine, cystine-homocystine disulfide, and methylmalonic acid promptly fell to normal after treatment with vitamin $B_{12}$. It is of interest that other compounds in the citric acid cycle—namely, α-ketoglutaric and *cis*-aconite acid—appeared in large amounts after treatment with parenteral $B_{12}$. This effect is being studied.

Further studies of the case of Dr. Shipman and his colleagues will be needed to prove their interesting hypothesis. In the meantime we wish to point out that significantly increased quantities of homocystine may be found in the urine of patients with pernicious anemia due to any one of several causes, and the homocystinuria may, as in the case we report here, be due to vitamin-$B_{12}$ deficiency.

This work was supported in part by NIH grant RR-61 and GRS grant FR-5365.

J. G. Hollowell, Jr.
W. Knowlton Hall
Margaret E. Coryell
James McPherson, Jr.
Dorothy A. Hahn

Departments of Pediatrics
and Biochemistry,
Medical College of Georgia,
Augusta, Georgia 30902.

**Defendant's Exhibit AW**

*Invited Review*

March-April 1977

Volume 273 Number 2

## Homocystinuria: pathogenetic mechanisms

ANTHONY J. GRIECO

Abstract: Homocystinuria with elevated plasma homocysteine and methionine levels is the result of deficient activity of cystathionine synthetase, the enzyme catalyzing conversion of homocysteine to cystathionine. It is inherited as an autosomal recessive trait with a worldwide distribution. The major clinical manifestations result from the elevated plasma homocysteine level. The excitotoxic effect of homocysteic acid accounts for mental retardation and seizures. Interference with collagen cross-linking by sulfhydryl groups of homocysteine causes ectopia lentis and skeletal deformities. Sulfation factor-like effects contribute to disruption of vascular endothelium, which is followed by platelet thrombosis and widespread arterial and venous occlusions. Low methionine homocystinuria, with deficient remethylation of homocysteine, results from deranged vitamin $B_{12}$ metabolism and from deficient 5,10-methylene-tetrahydrofolate reductase. Administration of azaribine produces homocystinuria by a mechanism not yet elucidated.

**Defendant's Exhibit BA**

The New England Journal of Medicine

Copyright, 1978, by the Massachusetts Medical Society

Volume 299 August 17, 1978 Number 7

# A SYNDROME OF METHYLMALONIC ACIDURIA, HOMOCYSTINURIA, MEGALOBLASTIC ANEMIA AND NEUROLOGIC ABNORMALITIES IN A VITAMIN $B_{12}$-DEFICIENT BREAST-FED INFANT OF A STRICT VEGETARIAN

Marilyn C. Higginbottom, M.D., Lawrence Sweetman, Ph.D., and William L. Nyhan, M.D., Ph.D.

Abstract: We studied a six-month old infant with severe megaloblastic anemia, coma and hyperpigmentation of the extremities. He was found to have methylmalonic aciduria (79 μmol per milligram of creatinine) and homocystinuria (0.85 μmol per milligram of creatinine) Additional biochemical abnormalities included cystathioninuria, glycinuria, methylcitric aciduria, 3-hydroxypropionic aciduria and formic aciduria. The concentration of vitamin $B_{12}$ in the serum was 20 pg per milliliter. This severe nutritional deficiency was a consequence of inadequate intake, for the infant was exclusively breast-fed by a strictly vegetarian mother who manifested methylmalonic aciduria. Our observations emphasize the importance of educating strict vegetarians about the deficiency of vitamin $B_{12}$ in their diets and the importance of vitamin $B_{12}$ supplementation. (N Engl J Med 299:317-323, 1978)

The increasing rarity of nutritional-deficiency disease may create problems in medical diagnosis and management for physicians in developed countries. Vegan diets – those from which animal proteins are strictly excluded – have become increasingly popular. Although rich in many vitamins, these

diets are deficient in vitamin $B_{12}$. In the infant described below, striking dysfunction of the hematopoietic and central nervous systems resulted from nutritional deficiency of vitamin $B_{12}$. The infant had been exclusively breast-fed by a strict vegetarian mother. The presentation of the infant suggested an inborn error of metabolism, and he was found to have methylmalonic aciduria and homocystinuria. Both the biochemical and the clinical abnormalities resolved after therapy with vitamin $B_{12}$. These observations emphasize the need for vitamin $B_{12}$ supplementation in vegan women who breast-feed their infants or for supplementation for the infants themselves.

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**Defendant's Exhibit BE**

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# Homocysteine in the plasma of renal transplant recipients: effects of cofactors for methionine metabolism

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**Summary**

1. Homocysteine which is formed during the metabolism of methionine is readily oxidized and is measured by the amino acid analyzer as cysteine-homocysteine mixed disulphide and homocystine. We measured plasma amino acid concentrations after an overnight fast in 27 stable long-term renal transplant recipients and 25 age and sex-matched normal subjects with particular emphasis on sulphur-containing amino acids.

2. Plasma cysteine-homocysteine mixed disulphide was increased in the patients (mean 6·0 ± SD 3·2 μmol/l; normal 3·1 ± 0·9 μmol/l. $P < 0·001$) and homocysteine was detectable in low concentration (<1·0 μmol/l) in 24; the elevation in cysteine-homocysteine was related to serum creatinine ($r = 0·60$. $P < 0·002$). Cystine was also increased (91·6 ± 29·3 μmol/l; normal subjects 64·0 ± 16·7 μmol/l, $P < 0·001$), but methionine concentrations were normal.

3. When pyridoxine, folic acid and vitamin $B_{12}$, cofactors for homocysteine metabolism, were administered sequentially to 11 arbitrarily selected transplant recipients cysteine-homocysteine decreased from 7·3 ± 2·1 to 4·3 ± 0·8 μmol/l ($P < 0·001$) and homocystine became undetectable.

The response coincided with the giving of folic acid and occurred without alteration in serum creatinine and with normal serum folate and vitamin $B_{12}$ concentrations.

4. In eight patients in whom pretreatment erythrocyte folate was measured, folic acid therapy reduced cysteine-homocysteine from 9·0 ± 3·1 to 5·4 ± 1·6 μmol/l over a 4 week period ($P < 0.001$), the largest response being in the one patient with subnormal erythrocyte folate; values were in the low-normal or normal range in the other seven.

5. We conclude that plasma homocysteine is increased in renal transplant recipients when serum creatinine is only moderately elevated and that the homocysteine concentrations are decreased by treatment with folic acid, suggesting that both reduced homocysteine excretion and relative shortages of folic acid are responsible.

Key words: amino acids, cysteine-homocysteine mixed disulphide, cystine, folic acid, homocysteine, homocystinuria, pyridoxine, renal failure, vascular disease.

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**Defendant's Exhibit BF**

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# Vitamin $B_{12}$ deficiency in a breastfed infant of a mother with pernicious anemia

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Nutritional vitamin $B_{12}$ (cobalamin) deficiency is rare in infants, although reports from Europe (Burgio *et al.* 1956. Gerbasi 1960) and India (Jadhav *et al.* 1962. Jathar *et al.* 1970) show that it can lead to severe megaloblastic anaemia together with neurological abnormalities. In America, infantile megaloblastic anaemia is more often the result of folate deficiency (Luhby 1959), though similar clinical effects have been observed in nutritional cobalamin deprivation (Higginbottom *et al.* 1978).

A case is now reported of a 14-month-old breastfed girl, presenting with severe neurological features, in whom the finding of cobalamin deficiency led to the diagnosis of pernicious anaemia in her mother. Treatment with hydroxocobalamin led to the child's complete recovery.

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*Investigations:*

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The routine blood biochemistry on admission was essentially normal. Results of haematological investigations

were as follows: Hb 9.4 g/dl. WBC 9.7 x $10^9$/l with a marked neutropenia platelets 564 x $10^9$/l. The blood film was macrocytic and bone marrow from the left posterior iliac crest was frankly megaloblastic. At this stage an EEG was markedly abnormal with gross generalized changes and a CAT brain scan showed abnormalities similar to those in diffuse cerebral atrophy. Methylmalonic acid (MMA) was first detected in the urine by routine chromatography and was later estimated as 1000 µg/mg creatinine (normal 0-5). Urinary cystathionine, homocystine and the mixed disulphide of cysteine-homocysteine also abnormally raised. While in the plasma levels of cystathionine and homocysteine were elevated but methionine was low. Taken together, these findings suggested a possible defect in cobalamin metabolism (Figure 1). The patient's serum total cobalamin was grossly low: 40 ng l by Euglena (normal 150-1000) and 46 ng l by radioisotopic assay (normal 130 950); total folate was also very low, serum folate being 0.4 µg l (normal 6.0-21) and red cell folate 11 µg l (normal 150 1000). In addition, her serum iron (6.2 µmol l: normal 6 16) and total iron binding capacity (57.8 µmol/l; normal 54 89) were at the lower limit of normal.

* * * *

Known metabolic defects of cobalamin metabolism are shown schematically in Figure 1. A cellular deficiency of MeCbl and AdoCbl leads to increased excretion of homocystine and MMA respectively. In the present case the low plasma level of MeCbl and total Cbl suggested either a dietary deficiency, malabsorption or a disorder of cobalamin transport. Selective cobalamin malabsorption (Imerslund-Gräsbeck syndrome) was unlikely since there was no proteinuria and a transport defect was excluded by the finding that TCI and TCII-binding were normal. Since the mother's milk contained only about one-tenth the lowest concentration of cobalamin normally present in breast milk and since this was the child's sole diet, a nutritional deficiency seemed the most likely. Unfortunately, the breast milk folate was not estimated, but both mother and child had low serum and red cell folate concentrations. Since SG was treated with folic acid simultaneously with OHCbl, one cannot be entirely

certain that a folate deficiency played no part in the progress of the disease. However, the finding of a low Schilling test and a haematological response to CNCbl in the mother strongly suggests that the child's primary deficiency was of cobalamins.

**Defendant's Exhibit BQ**

## Moderate Homocysteinemia – A Possible Risk Factor For Arteriosclerotic Cerebrovascular Disease

Lars E. Brattstrom, M.D., Jan Erik Hardebo, M.D. and Bjorn L. Hultberg, M.D.

SUMMARY Highly elevated concentrations of homocysteine measured as homocysteine or cysteine-homocysteine mixed disulfide (MDS) are found in plasma and urine in subjects with inherited abnormalities of the methionine metabolism. These subjects have a high incidence of arteriosclerotic vascular complications during childhood. Homocysteine causes endothelial cell injury and cell detachment that initiates the development of arteriosclerosis. The present study demonstrates a significantly elevated mean plasma MDS concentration in 19 patients with arteriosclerotic cerebrovascular disease compared to 17 controls. Our findings suggest that moderate homocysteinemia might be a risk factor for arteriosclerotic cerebrovascular disease.

Stroke Vol 15, No 6. 1984

HOMOCYST(E)INEMIA/URIA IS usually secondary to deficiency of cystathionine β-synthase, a pyridoxal phosphate dependent enzyme to the transsulfuration pathway (fig. 1). It is an autosomal recessive inborn error of methionine metabolism. Homocysteinemia can also be due to a reduced capacity for homocysteine remethylation via the folate and cobalamine dependent transmethylation pathway. It is characterized by elevated concentrations of homocysteine and its two disulfides homocystine and cysteine-homocysteine mixed disulfide (MDS) in plasma and urine.

The main cause of morbidity and morality in homocysteinemic patients is progressive premature arteriosclerosis and associated thromboembolic complications. Histopathologically there are widespread arterial focal lesions with fibrous intimal plaques and medial fibrosis with fraying and

splitting of the internal elastic membranes. Homocysteine or its derivates are considered to cause these changes.

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**Defendant's Exhibit BU**

## Folic Acid Responsive Postmenopausal Homocysteinemia

Lars E. Brattström. Björn L. Hultberg. and Jan Erik Hardebo

Homocysteinemia is associated with juvenile arteriosclerosis, recurrent thromboembolic complications and osteoporosis. Plasma homocysteine, measured as homocysteine-cysteine mixed disulfide (MDS), has in other than homocysteinemics been reported to be higher in patients with coronary heart or cerebrovascular disease than in controls, and higher in men than in premenopausal women. Here, in groups of normal men and normal premenopausal and postmenopausal women, we measured plasma MDS in the fasting state and four hours after a methionine load (100 mg/kg body weight), before and after four weeks of folic acid therapy at 5 mg daily. In their fasting plasma, postmenopausal women (n - 5) had significantly ($P < 0.05$) higher MDS concentrations than premenopausal women (n - 5) and younger men (n – 5). After the methionine load MDS concentrations in postmenopausal women rose markedly, reaching levels significantly higher than those in younger men ($P < 0.05$), and with no overlap with values in premenopausal women ($P < 0.01$), or in older men (n - 5, $P < 0.01$). Folic acid therapy resulted in substantial reductions (n - 15, $P < 0.01$) of MDS concentrations both before the methionine load (-31%) and after (-28%), though subjects had initially had normal concentrations of serum and erythrocyte folates. We speculate that moderate homocysteinemia might contribute to postmenopausal arteriosclerosis and osteoporosis. Should this prove to be the case, folic acid might be a useful prophylactic.

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METHIONINE, an essential amino acid containing sulfur is metabolized to cysteine by means of the transsulfuration pathway. Homocysteine constitutes the juncture between this pathway and the folate, cobalamin, and betaine dependent transmethylation cycle (Fig 1). Deficiencies of certain

enzymes involved in these pathways, will result in homocysteinemia, owing to the accumulation, in plasma and tissue, of homocysteine and its two disulfides–homocystine (homocysteine-homocysteine) and homocysteine-cysteine mixed disulfide (MDS).

Homocysteinemia is associated with juvenile arteriosclerosis, recurrent arterial and venous throboembolic manifestations, and osteoporosis. If untreated, more than 50% of affected individuals die before the age of 20 of myocardial infarction, stroke or pulmonary embolism. Experimental homocysteinemia in baboons has shown that homocysteine induces endothelial injury, arteriosclerosis, and thrombosis. Homocysteine also interacts with collagen synthesis, an interaction that may be significant in the development of osteoporosis.

Homocysteine concentrations, measured as MDS in plasma from men with ischemic heart disease and controls have been investigated in two studies by Wilcken et al. in one of which, though not in the other, they found significantly higher MDS concentrations in patients than in controls after a methionine load. They suggested that persistent moderate increases in plasma homocysteine might be a risk factor for arteriosclerotic coronary heart disease. In a recent study we measured plasma MDS in patients with carotid artery stenosis, TIAs or strokes, and found significantly higher ($P < 0.05$) concentrations in the patients both before and after a methionine load: female controls (> 50 years) tended to have higher values than males and younger females: three female controls were also given folic acid, 5 mg daily for four weeks, and then reinvestigated, a substantial reduction of the MDS concentrations being found in all three subjects both before and after the methionine load.

Before this study was begun, MDS concentrations in normal women and men had only been reported in young subjects, and to be significantly higher in men than in women. We therefore measured plasma MDS in groups of normal premenopausal and postmenopausal women, as well as in age-matched groups of normal men. The subjects were

investigated before and after a methionine load and reinvestigated after four weeks of folic acid therapy.

**Defendant's Exhibit CN**

**CONFIDENTIAL**

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January 5, 1990

Dr. Malinow
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Dear Dr. Malinow:

I enclose reprints of the work of Dr. Robert Allen and myself diagnosing vitamin B12 deficiency using serum levels of methylmalonic acid and homocysteine. The article in *Laboratory Management* describes the clinical problem in diagnosing this deficiency. It is not appropriate at the present time to state that because a patient has a serum cobalamin level >200 pg/ml, he has no chance of having B12 deficiency and, conversely, not every patient with a B12 level <200 pg/ml has B12 deficiency. Because of past literature showing that patients who receive folic acid in pharmacologic amounts who actually have vitamin B12 deficiency may have progression of neuropsychiatric deficits, I feel that a shotgun approach of giving folic acid to every patient with an elevated serum homocysteine is clearly not warranted. It can be difficult to specifically make the diagnosis of B12 deficiency in a patient with slight hematologic abnormalities or neuropsychiatric abnormalities

without associated hematologic abnormalities. I feel that any patient who had an elevated homocysteine should certainly have a methylmalonic acid level performed since if both levels are elevated, the patient has vitamin B12 deficiency or chronic renal failure. If a patient has isolated homocysteine elevation, then the possibilities still include B12 deficiency, occult alcoholism or antifol treatment (numerous drugs interfere with folate metabolism, i.e. dilantin, tegretol, methotrexate, sulfa drugs, etc.). It would seem likely that the heterozygous state for cystathionine beta synthase deficiency would probably be less represented in the general population than these other clinical situations which would also cause an increase in serum homocysteine. This would be particularly true in elderly populations since, as shown in our preliminary data in the enclosed abstract (submitted to the American Geriatrics Society), there is a high prevalence of unsuspected vitamin B12 deficiency in elderly outpatients, perhaps as high as 14%.

We plan to discuss studies on standardizing the homocysteine assay with Dr. Refsum at the Vermont meeting of B12, folate and 1-carbon metabolism this summer, assuming she will attend.

Sincerely,

Sally P. Stabler, M.D.

**CONFIDENTIAL**

**Defendant's Exhibit IJ**

**CONFIDENTIAL**

# HYPERHOMOCYSTEINEMIA: A COMMON AND TREATABLE CAUSE OF VASCULAR DISEASE AND NEURAL TUBE DEFECTS

*Medical Grand Rounds — September 13, 1995*

SALLY P. STABLER, M.D.

Learning Objectives

1. Know that homocysteine is a sulfhydryl-containing amino acid and it's metabolic role.

2. Know that homocysteine levels depend on vitamin $B_{12}$, folate, $B_6$ or renal status.

3. Learn important aspects for critiquing in vivo and in vitro studies of hyperhomocysteinemia.

4. Be aware that genetic polymorphisms may interact with environmental factors to produce hyperhomocysteinemia.

* * * *

Homocysteine Metabolism

Homocysteine can be methylated to form methionine by methyltetrahydrofolate in a reaction requiring methyl-$B_{12}$ as a cofactor or condensed with serine to form cystathionine which is a precursor of cysteine in reactions that are dependent on vitamin $B_6$ (pyridoxical-5'-phosphate). The former reactions are referred to as transmethylation and the latter as transsulfuration. The pathway below shows the structures of the compounds involved.

* * * *

Hyperhomocysteinemia

Hyperhomocysteinemia is a term used to describe elevated total homocysteine levels in serum or plasma. The term homocystinuria is reserved for the genetic disorder caused by a deficiency of cystathionine-B-synthase. Hyperhomocysteinemia can be caused by deficiencies of vitamin $B_{12}$ and folate and possibly vitamin $B_6$, drugs that interfere with folate, $B_6$ or $B_{12}$ metabolism, by congenital defects of the enzymes involved in folate, $B_{12}$ and $B_6$ related enzymes, and by chronic renal insufficiency. Vitamin $B_{12}$ is a required cofactor in another pathway which converts L-methylmalonyl CoA to succinyl CoA. In defects in vitamin $B_{12}$ metabolism, methylmalonic acid levels increase together with serum total homocysteine (Stabler 1986, Allen 1993). If methylmalonic acid levels are increased with total homocysteine then the defect is related to $B_{12}$ metabolism versus folate metabolism. Even very mild degrees of renal failure can lead to increases in both serum total homocysteine and methylmalonic acid, and subjects with ESRD and those on dialysis have fairly marked elevations of homocysteine. The tables below list the common defects related with hyperhomocysteinemia.

| DRUGS | VITAMINS | GENETIC DEFECTS | MISCELL-ANEOUS |
|---|---|---|---|
| Anti-folate drugs | Vitamin $B_{12}$ Deficiency | Cystathionine B-synthase Deficiency (homocystinuria) | Renal Insufficiency |
| Methotrex-ate | Folate Deficiency | Vitamin $B_{12}$ Defects in: | Psoriasis |
| Pyrimeth-amine | Vitamin $B_6$ Deficiency | Absorption | Leukemia |
| Sulpha Drugs | | Transport | |
| Triam-terene | | Coenzyme formation | |
| Phenytoin | | Methionine Synthase Defects | |
| Carbam-azepine | | Folate Defects in: | |
| Alcohol | | Absorption | |
| $N_2O$ | | Transport | |
| | | 5,10-Methylenete-trahydrofolate Reductase Deficiency | |

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**Defendant's Exhibit JB**

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# HOMOCYSTEINE AND CARDIOVASCULAR DISEASE

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KEY WORDS: homocysteine, vascular disease, folic acid, vitamin B12, vitamin B6

## ABSTRACT

An elevated level of total homocysteine (tHcy) in blood, denoted hyperhomocysteinemia, is emerging as a prevalent and strong risk factor for atherosclerotic vascular disease in the coronary, cerebral, and peripheral vessels, and for arterial and venous thromboembolism. The basis for these conclusions is data from about 80 clinical and epidemiological studies including more than 10,000 patients. Elevated tHcy confers a graded risk with no threshold, is independent of but may enhance the effect of the conventional risk factors, and seems to be a particularly strong predictor of cardiovascular mortality. Hyperhomocysteinemia is attributed to commonly occurring genetic and acquired factors including deficiencies of folate and vitamin B12. Supplementation with B-vitamins, in particular with folic acid, is an efficient, safe,

and inexpensive means to reduce an elevated tHcy level. Studies are now in progress to establish whether such therapy will reduce cardiovascular risk.

## INTRODUCTION

The total homocysteine (tHcy) level in plasma or serum is a sensitive indicator of vitamin B12 and folate deficiencies (1). It is related to pregnancy complications (2), neural tube defects (3), mental disorders (4, 5), and cognitive impairment in the elderly (6, 7). Furthermore, data from about 80 clinical and epidemiological studies provide ample evidence that an elevated tHcy level is a common cardiovascular risk factor in the general population (8-10).

### *Determinants of the tHcy Level*

Determinants of tHcy include genetic and acquired factors as summarized in Table 1.

AGE, SEX, AND RENAL FUNCTION Women have lower tHcy than men, and tHcy increases with age (37, 53, 54). This may partly be due to differences in vitamin status (55), but also to the influence of sex hormones. Plasma tHcy, levels increase after menopause (53, 56), which may explain the steeper age-related increase in women compared with men (54). The sex difference may also be related to stoichiometric formation of Hcy in connection with the creatine/ creatinine synthesis that is proportional to muscle mass, and therefore higher in men than in women (57).

Renal function is a strong determinant of the tHcy level (58, 59). This is probably related to Hcy clearance via renal metabolism (60) rather than urinary excretion, which is minor (52, 61). The physiologic decline in renal function may partly explain the age effect (62, 63).

LIFESTYLE Dietary intake of vitamin B6, B12, and folate is inversely correlated to plasma tHcy (55). Smoking and caffeinated coffee consumption cause a shift of the distribution towards higher tHcy values, whereas physical activity is associated with low tHcy levels (54, 64). Notably, the effect of these lifestyle factors on the tHcy level seems to be more

pronounced in women than in men (37, 54, 64). Chronic, high ethanol consumption is associated with elevated tHcy levels (65), possibly through its effect on vitamin status. In contrast, a moderate consumption seems to be associated with a lower tHcy level (66).

GENETIC DETERMINANTS Homocystinuria usually refers to the inborn errors of Hcy metabolism associated with severe hyperhomocysteinemia. Homozygosity for CBS deficiency is the most common cause, with a birth prevalence of 1/300,000, but with marked geographic differences (15). Rare forms of homocystinuria include severe defects of MTHFR (67) and impaired Hcy remethylation due to inborn errors of cobalamin metabolism (68).

Heterozygosity for CBS deficiency is present in < 1 % of the general population (15). These subjects usually have a normal fasting tHcy level, but the PML tHcy level may be elevated (43, 44). Recent genetic studies have shown that heterozygosity for CBS deficiency occurs only sporadically in vascular patients (14, 69, 70) and suggest that this mild genetic defect is not a frequent cause of hyperhomocysteinemia in these patients.

The common C677T mutation in the MTHFR gene shows ethnic differences with a high allele frequency of about 40% (10% TT genotype) in Caucasians (71), whereas it is almost absent in African-Americans (72). This polymorphism causes reduced enzyme activity and thermolability and predisposes to moderate (15-30 μM) and intermediate (30-100 μM) hyperhomocysteinemia under conditions of impaired folate status (29, 73, 74). A low daily dose (0.2 mg) of folic acid will probably enable maintenance of a normal tHcy level in most of these subjects (73).

**Table 1 Determinants of the plasma total homocysteine level**

| | Effect | Reference(s) |
|---|---|---|
| Genetic factors | | |
| Homozygosity for CBS defects | ↑↑↑ | 15.80 |
| Homozygosity for MTHFR defects | ↑↑↑ | 81.82 |
| Cobalamin mutations (C, D, L, I, G) | ↑↑↑ | 68.83 |
| Down's syndrome | ↓ | 84 |
| Thermolabile MTHFR | ↑ | 14, 73, 74 |
| Heterozygosity for CBS defects[1] | ↑ | 85 |
| Heterozygosity for MTHFR defects | ↑ | 81, 82 |
| Physiologic determinants | | |
| Increasing age | (↑) | 53, 54 |
| Male sex | (↑) | 53, 54 |
| Renal function, reduced GFR | (↑) | 86 |
| Increasing muscle mass | (↑) | 57 |
| Lifestyle determinants | | |
| Vitamin intake | ↓ | 55 |
| Smoking | (↑) | 54 |
| Coffee consumption | (↑) | 64 |
| Ethanol consumption | ↑↓ | 65, 66 |
| Physical activity | ↓ | 54 |
| Clinical conditions | | |
| Folate deficiency | ↑↑ | 1, 75 |
| Vitamin B12 deficiency | ↑↑↑ | 1 |
| Vitamin B6 deficiency[1] | ↑ | 87 |
| Renal failure | ↑↑ | 59, 76 |
| Hyperproliferative disorders | ↑ | 88, 89 |
| Hypothyroidism | ↑ | 90 |
| Drugs | | |
| Folate antagonists (methotrexate) | ↑ | 77 |

| | | |
|---|---|---|
| Vitamin B12 antagonists (nitrous oxide) | ↑↑ | 91, 92 |
| Vitamin B6 antagonists[1] | ↑ | 45, 93 |
| AdoHcy hydrolase inhibition | ↓ | 94 |
| Antiepileptic drugs | ↑ | 8 |
| Contraceptives, hormone therapy | ↓ | 95-97 |
| Aminothiols (acetylcysteine, penicillamine) | ↓ | 78, 79 |
| Others (L-dopa, cholestyramine, niacine) | ↑ | 98-100 |

↓ = Reduction of the total homocysteine level; (↑) = increase within normal reference range; ↑, ↑↑, ↑↑↑ = moderate hyperhomocysteinemia (15-30 μM), intermediate hyperhomocysteinemia (30-100 μM), and severe hyperhomocysteinemia (>100 μM), respectively. AdoHcy = S-adenosylhomocysteine; CBS = cystathionine β-synthase; MTHFR = methylenetetrahydrofolate reductase.

[1]In subjects with vitamin B6 deficiency or mild defects in CBS, the fasting total homocysteine level is usually normal but the postmethionine load level is increased.

CLINICAL CONDITIONS AND DRUGS Folate or cobalamin deficiency are common causes of hyperhomocysteinemia in the general population (1, 75). Elevated tHcy levels are also observed in renal failure (59, 76), and in various other clinical conditions (Table 1) (9). Hyperhomocysteinemia is induced by certain drugs, especially those affecting the vitamins related to Hcy metabolism (77). Aminothiols, like penicillamine (78) and acetylcysteine (79), reduce the plasma tHcy level.

*Reference Ranges*

FASTING tHcy The normal range in adults is usually 5-15 μM, with a mean level of about 10 μM (Table 2) (32). Hyperhomocysteinemia is defined as a plasma tHcy > 15 μM and is denoted as moderate (15-30 μM), intermediate (30-100 μM) and severe (> 100 μM) hyperhomocysteinemia (101).

Reported reference ranges differ markedly (32). These variations are probably related to a skewed tHcy distribution toward higher values, varying defintions of the upper threshold, differences in sample handling and methodology, and variations in determinants of the (tHcy levels among different populations).

It has been suggested that reference ranges should be determined in a population with adequate vitamin status (102-104). Such individuals have markedly lower tHcy levels (with an upper limit of about 12 μM) than the general population. The 2.5-97.5 percentiles for 40-42-year-old subjects in the Hordaland population (n = 18,043) were 5.6-17.4 μM for women and 6.9-20.9 μM for men. The corresponding values for non-smokers with high folate intake and low coffee consumption were 4.7-11.4 μM and 6.3-13.1 μM, respectively (105).

Only sparse data exist on tHcy levels in children (88, 106-108). Recently, Tonstad et al found that tHcy levels in 8-12-year-old boys and girls are about half of that observed in adults. There was no sex difference, and the tHcy frequency distribution was nearly gaussian, with a mean tHcy level of 5.25 μM and a reference range (mean ± 2SD) of 2.9-7.6 μM (107). At puberty, tHcy increases markedly, and the distribution becomes skewed as in adult populations (108 ).

PML tHcy Relatively few investigations have addressed the reference range for PML tHcy levels (48, 53, 109, 110), and various definitions for an abnormal response have been used (9). Relative to the fasting level, the PML tHcy and the increase in tHcy measured after 4 to 6 h are usually about 3 and 2 times higher, respectively (Table 2). PML tHcy is higher in men than in women and increases with age, especially in women (109, 110).

**Table 2 Fasting, postmethionine load (PML) and increase after loading in plasma total homocysteine level in the control group of the European Concerted Action Project on homocysteine and vascular disease (49)**

| | Percentile | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2.5 | 5 | 10 | 20 | 50 | 80 | 90 | 95 | 97.5 |
| | (μmol/L) | | | | | | | | |
| **All (n=800)** | | | | | | | | | |
| Fasting | 5.9 | 6.4 | 7.0 | 7.7 | 9.5 | 12.1 | 13.4 | 15.7 | 18.5 |
| PML | 17.4 | 19.2 | 21.5 | 24.2 | 29.9 | 38.0 | 43.7 | 51.3 | 58.1 |
| Increase | 10.4 | 11.6 | 13.1 | 15.4 | 19.8 | 27.3 | 31.6 | 37.2 | 42.8 |
| **Men < 45 years (n=232)** | | | | | | | | | |
| Fasting | 6.3 | 6.8 | 7.2 | 7.8 | 9.6 | 11.9 | 12.9 | 15.8 | 18.8 |
| PML | 17.3 | 19.0 | 20.9 | 23.8 | 29.6 | 39.8 | 47.5 | 56.4 | 60.9 |
| Increase | 10.4 | 11.4 | 12.3 | 15.2 | 19.3 | 28.2 | 34.1 | 39.1 | 44.7 |
| **Women < 45 years (n=135)** | | | | | | | | | |
| Fasting | 4.8 | 5.3 | 6.0 | 6.6 | 8.1 | 10.4 | 12.6 | 13.6 | 16.9 |
| PML | 16.0 | 16.6 | 17.9 | 20.0 | 26.0 | 34.1 | 40.2 | 44.7 | 48.2 |
| Increase | 8.9 | 9.9 | 11.3 | 13.1 | 17.8 | 23.9 | 30.1 | 32.1 | 34.9 |
| **Men > 45 years (n=338)** | | | | | | | | | |
| Fasting | 6.8 | 7.2 | 7.8 | 8.5 | 10.3 | 12.6 | 13.7 | 15.9 | 18.6 |
| PML | 21.0 | 22.0 | 23.6 | 25.7 | 31.0 | 38.0 | 42.9 | 50.3 | 57.9 |
| Increase | 12.8 | 13.8 | 14.9 | 16.2 | 20.6 | 26.6 | 30.1 | 35.5 | 41.7 |
| **Women > 45 years (n=95)** | | | | | | | | | |
| Fasting | 5.8 | 6.3 | 6.7 | 7.1 | 9.1 | 11.4 | 12.9 | 13.7 | 15.1 |
| PML | 16.8 | 19.6 | 21.5 | 24.0 | 31.2 | 36.9 | 42.4 | 50.1 | 54.2 |
| Increase | 10.1 | 12.0 | 13.7 | 15.3 | 21.4 | 28.2 | 33.3 | 40.3 | 43.2 |

## HOMOCYSTEINE AND CARDIOVASCULAR DISEASE

*History and Summary of Highlights up to 1995*

Homocystinuria was described in 1962 in mentally retarded children (111, 112). About two years later, the defect in CBS was identified (113), and it was reported that such patients frequently had thromboembolic events (114, 115). Severe MTHFR deficiency (67, 116) and certain defects in intracel-

lular cobalamin metabolism (68, 117) were later reported to cause a similar clinical picture. In 1969, McCully described the vascular pathology of homocystinuria (117). He noted that thromboembolic disease was a characteristic feature of homocystinuria independent of the site of the metabolic defect, pointing to Hcy as the causal agent. This is the basis for his Hcy theory of atherosclerosis, which implies that moderately elevated tHcy may be a cardiovascular risk factor in the general population (118).

In 1976, Wilcken & Wilcken published the first report that coronary artery patients frequently have abnormal Hcy metabolism (119). For the following 15 years, there were only scattered reports (8, 9) on the relation between Hcy levels and coronary artery disease (120-122), cerebrovascular disease (123-125), peripheral artery disease (124, 126, 127), and venous thrombosis (128, 129). In the same period, the most important determinants of tHcy were identified, including age and sex (121, 130), renal function (76, 131), and folate and vitamin B12 status (75, 132). In 1988, Kang et al reported on thermolabile MTHFR and its relation to cardiovascular risk and hyperhomocysteinemia (27, 28).

Since 1990, there has been an exponential increase in the publication rate on tHcy and cardiovascular disease (Figure 2). This is related to the recognition of elevated tHcy as an independent cardiovascular risk factor (169). But equally important was the introduction of various assays for tHcy determination (32), which are more practical in the clinical setting and allow the use of stored blood samples. The first positive prospective studies on tHcy and coronary heart disease were reported in 1992 (170) and 1993 (171). One negative prospective study on myocardial infarction and stroke from Finland (172) caused some concern about the validity of the Hcy theory, but the hypothesis was later substantiated by several reports which indicated that tHcy is a risk factor for cardiovascular disease. In 1995, Selhub et al reported on a strong relation between tHcy and extracranial carotid-artery stenosis in the elderly (173), and a prospective study demonstrated that tHcy conveyed a graded risk for stroke in middle-aged British men (162). The same year, the

C677T mutation in the MTHFR gene was identified and proposed as a candidate risk factor for vascular disease (29).

In 1995, Boushey et al reviewed most studies on Hcy and cardiovascular disease. Their meta-analysis, based on 27 studies including about 4000 patients, showed that Hcy was an independent, graded risk factor for atherosclerotic disease in the coronary, cerebral and peripheral vessels (10). Since then, there have been about 40 additional studies on tHcy as a risk factor for cardiovascular disease and its complications (Table 3): the majority of them support the conclusions formulated by Boushey et al. The strongest evidence derives from ten prospective studies: Eight demonstrate an increase in risk of stroke (162, 174), coronary heart disease (163, 170), venous thrombosis (164), cardiovascular complications (166, 167) or mortality (168), whereas only two are negative (165, 172). Recent data have been reviewed and subjected to frequent editorial comments (175-179).

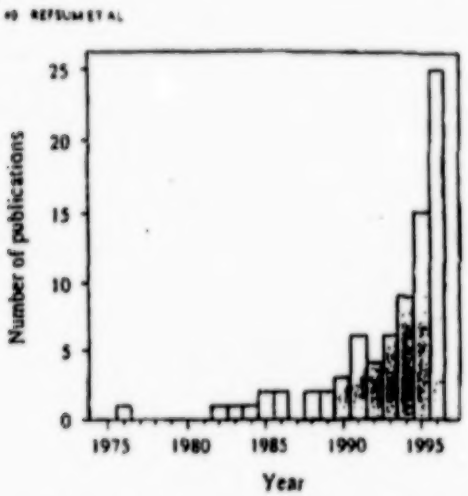



*Figure*. Number of clinical and epidemiological studies on homocysteine and cardiovascular disease in the years 1976 to 1996. Nine publications on methylenetetrahydrofolate and cardiovascular disease are included.

*Hcy and Conventional Risk Factors*

Hcy is one of more than 200 identified risk factors for cardiovascular disease (180). Thus, a frequently asked

question is whether the reported tHcy association is due to confounding with established factors.

A correlation between tHcy level and total, HDL or LDL cholesterol has been shown in some studies (9, 54, 62, 135, 163, 181). Two recent studies have smoking habits, blood pressure (Figure 3), and serum creatinine. Our data suggest that an increased tHcy level may contribute to acute thromboembolic events leading to death.

*Mechanisms*

Observations in homocystinuria patients, animal experiments, and in vitro studies have identified several potential sites where hyperhomocysteinemia may induce vascular lesions. These targets include connective tissue and smooth-muscle cells, platelets, endothelial cells, the vessel wall, blood lipids, coagulation factors, and nitric oxide.

In vitro (222) and in vivo (223) experiments suggest that Hcy promotes aggregation of platelets, but this has been contested (224, 225). Endothelial damage mediated through $H_2O_2$ production has been proposed (226-229), but cysteine induces similar effects, and the validity of these observations for in vivo atherogenesis has been questioned (23). Hcy increases DNA synthesis, growth, and cyclin A gene expression (231) in cultured vascular smooth muscle cells, and cyclin-dependent kinase expression in the aorta of rats (232). Oxidative modification of LDL by Hcy has been demonstrated in vitro (233, 234) but has not been observed in hyperhomocysteinemic patients (235, 236). Physiologic levels of Hcy may enhance the binding of lipoprotein(a) to fibrin (237), but cysteine and other thiols have a similar effect. High concentrations of Hcy in vitro activate factor V (238), reduce protein C activation (239, 240), inactivate the cofactor activity of thrombomodulin (241), suppress thrombomodulin (240) and anticoagulant heparan sulfate expression (242), and block tissue plasminogen activator binding to human endothelial cells (243). Hcy rapidly reacts with endothelium-derived relaxing factor/nitric oxide (NO) to form S-nitroso-Hcy, which acts as a potent antiplatelet agent and vasodilator. The formation of this adduct may attenuate

$H_2O_2$ production from Hcy and, thereby, protect against the atherogenic properties of Hcy. According to this model, vascular injury is caused by an imbalance between NO production from dysfunctional endothelial cells and the levels of Hcy (225). Notably, impaired endothelium-dependent vasodilatation associated with elevated tHcy has been demonstrated in vivo (244, 245). The possible etiologic mechanisms of Hcy in vascular disease have been summarized in recent review articles (225, 246).

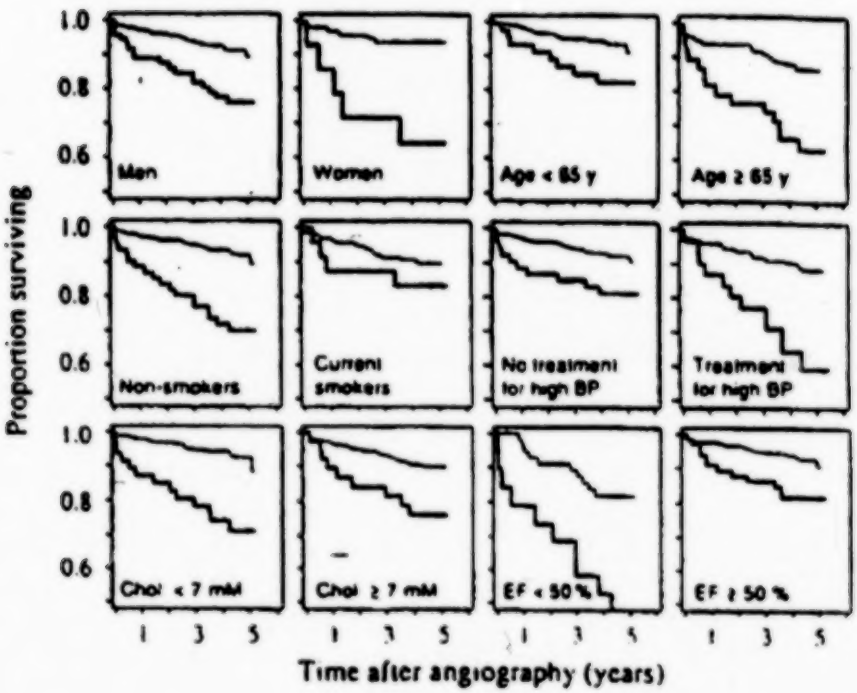



*Figure 3* The relation between plasma total homocysteine (tHcy) and mortality Kaplan-Meier survival plots comparing patients with tHcy below (thin line) and above (thick line) 15 μM in various subgroups. The tHcy level was measured at the time of the angiography (168) BP, blood pressure; Chol., serum total cholesterol; EF left ventricular ejection fraction.

The data cited above show that there is no unifying hypothesis explaining the atherogenic and thrombogenic effects of circulating Hcy. This may reflect its diversity of effects,

but may also be due to flaws in study design or interpretation of data. In general, it is difficult to mimic the slow atherosclerotic effect by short-term in vitro studies. Another problem is related to the high millimolar concentrations of Hcy frequently used (229, 239, 243, 247, 248) that are 100- to 1000-fold higher than observed in moderate hyperhomocysteinemia (249). Moreover, the complex redox reactions involving the various Hcy forms and their relation to other aminothiols in vivo (31) contrast to the in vitro testing of a single Hcy species (249). Finally, the specificity of some Hcy effects should be questioned because they can be obtained by other thiols (230, 233, 250), in particular cysteine, which is the most abundant aminothiol in plasma, with a total concentration about 25-fold higher than tHcy (31).

## HOMOCYSTEINE-LOWERING THERAPY

Increased intake of folic acid, vitamin B12, and B6 will probably reduce the tHcy level in nearly all individuals independent of their pretreatment tHcy level (251). Vitamin therapy partly prevents the vascular complications of homocystinuria (15), and use of vitamins is associated with lower risk of vascular disease in the general population (49). A high intake of fruits and vegetables, which are good sources of dietary folate, protects against cardiovascular disease (252), and an observational study suggests that vitamin B6 may delay the progress of coronary heart disease (253). However, it remains to be shown in randomized placebo-controlled clinical trials that a reduction of the tHcy level has an overall beneficial effect.

### *Vitamin and Drug Therapy*

FOLIC ACID AND FOLATE INTAKE In 1988, Brattström et al showed that healthy subjects responded to a high does of folic acid (5 mg/d) with a marked reduction in their tHcy levels (254). Since then, several studies have demonstrated that 0.65-10 mg/d of folic acid alone or together with vitamin B12 and/or B6 reduce the fasting and PML tHcy level by 25-50%, both in healthy and in hyperhomocysteinemic subjects and in vascular patients (251, 255-257). Data on dose response are sparse, but no difference was found for 2.5

versus 10 mg/d inpatients with myocardial infarction (145) or for 0.5 versus 5 mg/d in healthy subjects (109).

Generally, a total folate intake from food and supplements below 200-250 μg/d is occasionally associated with hyperhomocysteinemia, whereas an intake of 300-400 μg/d usually ensures a normal to low tHcy level in the majority of the population (55, 258-260). A higher dose may be required to obtain a maximal tHcy reduction in subgroups such as patients with reduced renal function (261) or subjects with low folate status (73).

Folic acid is considered nontoxic and well tolerated for chronic use in high doses (262), but may mask the symptoms of vitamin B12 deficiency (see below).

VITAMIN B12 The effect of vitamin B12 on the tHcy level is modest with maximum 10-15% reduction (109, 254, 256), except in vitamin B12-deficient subjects (1). However, a low serum B12 level may prevent an optimal response to folic acid (73, 251). Moreover, there is high prevalence of vitamin B12 deficiency in elderly subjects (263). The concern that folic acid supplementation may alleviate hematological signs of B12 deficiency or even precipitate neuropathy has been thoroughly debated (10, 264-266). A daily vitamin B12 supplement of 200 μg/d can probably prevent the clinical symptoms of pernicious anemia (267).

VITAMIN B6 Oral treatment with pyridoxine up to 300 mg/d does not lower the fasting tHcy level in healthy subjects of vascular patients (251, 254, 257, 268). However, pyridoxine (10-250 mg/d) lowers an abnormal PML tHcy level in most patients, and, when combined with folic acid, nearly all obtain a normal PML tHcy level (45, 127, 255, 257, 269). Chronic use of vitamin B6 may precipitate peripheral neuropathy, but a daily dose of 100 mg or less is probably safe (270).

**Defendant's Exhibit JC**

AHA SCIENCE ADVISORY

# Homocyst(e)ine, Diet, and Cardiovascular Diseases A Statement for Healthcare Professionals From the Nutrition Committee, American Heart Association

M. René Malinow, MD; Andrew G. Bostom, MD; Ronald M. Krauss, MD

## Homocysteine and Diet

Homocysteine is a sulfur-containing amino acid, rapidly oxidized in plasma to the disulfides homocystine and cysteine-homocysteine (Figure 1). Plasma/serum total homocysteine, also termed homocyst(e)ine, is the sum of homocysteine in all 3 components. Figure 2 displays factors involved in the metabolism of homocysteine, including its metabolic relationship to methionine. Although dietary intake of total protein and methionine does not correlate significantly with blood homocyst(e)ine, a single dose of oral methionine (100 mg/kg body weight) can elevate homocyst(e)ine levels, and as described further below, this has been used as a diagnostic test to detect disordered homocyst(e)ine metabolism. Because variable changes in homocyst(e)ine levels have been observed postprandially, it is customary to obtain measurements in the fasting state. Normal levels of fasting plasma homocyst(e)ine are considered to be between 5 and 15 μmol/L. Moderate, intermediate, and severe hyperhomocyst(e)inemia refer to concentrations between 16 and 30, between 31 and 100, and >100 μmol/L, respectively.

Several vitamins function as cofactors and substrates in the metabolism of methionine and homocysteine (Figure 2). Folic acid and cyanocobalamin (vitamin $B_{12}$) regulate metabolic pathways catalyzed by the enzymes methylenetetrahydrofolate reductase (MTHFR) and methionine synthase, respectively, whereas pyridoxine (vitamin $B_6$) is a cofactor for cystathionine β-synthase. A number of studies have

shown inverse relationships of blood homocyst(e)ine concentrations with plasma/serum levels of folic acid, vitamin $B_6$, and vitamin $B_{12}$.

Administration of supplemental folic acid in doses between 0.2 and 15 mg/d can lower plasma homocyst(e)ine levels without apparent toxicity. On the basis of meta-analysis of 12 clinical studies; all but 1 of which was a placebo-controlled trial, it has been estimated that a 25% reduction in homocyst(e)ine concentration can be achieved with mean supplementation of 0.5 to 5.7 mg of folic acid per day; an additional 7% lowering has been observed after the addition of vitamin $B_{12}$ (0.02 to 1 mg/d; mean, 0.5 mg). A recent report of the Food and Nutrition Board of the Institute of Medicine has recommended an upper limit of 1 mg/d folic acid on the basis of the possibility that higher doses may mask signs of vitamin $B_{12}$ deficiency in some subjects. In overt cobalamin deficiency with intermediate and severe hyperhomocyst(e)inemia, vitamin $B_{12}$ can normalize homocyst(e)ine concentration in ~70% of cases. In an open-label, uncontrolled study, vitamin $B_6$, at $\leq$250 mg/d was without effect in reducing basal homocyst(e)ine levels, but doses of 50 to 250 mg/d reduced homocyst(e)ine levels after a methionine-loading test by ~25%. Subsequently, a study that used a randomized, placebo-controlled, 2x2 factorial design demonstrated that 50 mg of vitamin $B_6$ per day independently reduced the post-methionine-loading increase in homocyst(e)ine levels by 22%. In a placebo-controlled study, a combination of multiple agents including folic acid (0.65 mg/d), vitamin $B_6$ (10 mg/d), and vitamin $B_{12}$ (0.4 mg/d) was very effective in reducing homocyst(e)ine levels in patients with moderate or intermediate hyperhomocyst(e)inemia. It has been reported, however, that increased vitamin intake from food sources (1 mg of folic acid, 12.2 mg of pyridoxine, and 50 µg of cyanocobalamin per day) failed to maintain normal homocyst(e)ine levels attained previously by vitamin supplementation.

* * * *

## Vitamin Intake, Homocyst(e)ine, and Cardiovascular Disease

In the case-control study of arterial diseases described above, folate deficiency was more common in cases, and plasma vitamin $B_6$ below the lowest 20th percentile (<23.3 nmol/L) for control subjects was associated with increased risk for vascular disease, ie, OR=1.84 (95% CI, 1.39 to 2.42). Of note, the relationship between vitamin $B_6$ and vascular disease was shown to be independent of homocyst(e)ine levels. Morrison et al demonstrated in the prospective Nutrition Canada Survey that the risk of fatal CAD was associated with low serum folate in 165 CAD deaths in subjects among 5056 men and women monitored for 20 years.

In a subset of subjects from the Atherosclerosis Risk In Communities study, carotid artery medial-intimal thickening was associated with high levels of homocyst(e)ine. Moreover, in a subset of subjects from the Framingham Heart Study, the stenosis was inversely proportional to reported intakes of both folic acid and vitamin $B_6$. Another analysis from the Framingham Study indicated that intake of fruits and vegetables was inversely related to incidence of stroke over a 20-year follow-up. Although homocyst(e)ine levels were not measured, the authors discussed, among other factors, the potential role of dietary folic acid in lowering homocyst(e)ine as a plausible mechanism involved in the protective effects of diet.

Users of multivitamins have been reported to have a reduced prevalence of CAD compared with nonusers. These findings have been extended in prospective observations conducted by Rimm et al in 80 082 women from the Nurses' Health Study. During a 14-year follow-up, the risk for fatal and nonfatal CAD was considerably lower among women who used multivitamins 4 to 7 times per week than among nonusers (risk ratio=0.76; 95% CI, 0.65 to 0.90) after multivariate adjustments. Caution must be exercised in interpreting observational studies, however, because of the possible effects of differences in unmeasured behaviors or risk factors that may be associated with

diet and vitamin intake. Moreover, the relationship of vitamin use to homocyst(e)ine levels was not evaluated in the study by Rimm et al.

## Dietary Guidance With Regard to Homocyst(e)ine and Cardiovascular Disease

### Population Guidelines

On the basis of the apparent relationship of plasma homocyst(e)ine to cardiovascular disease risk and the estimated influence of folic acid on homocyst(e)ine levels, Boushey et al suggested that a 350 μg/d increase in folic acid intake in men and a 280 μg/d increase in women could potentially prevent 30 500 and 19 000 vascular deaths annually in men and women, respectively. However, in the absence of prospective, placebo-controlled intervention trials of the effects of diet- or vitamin-mediated homocyst(e)ine reductions on incidence of cardiovascular disease, the clinical benefits of such interventions are unknown.

A recent report of the Food and Nutrition Board of the Academy of Sciences Institute of Medicine includes RDAs for folic acid, vitamin $B_6$, and vitamin $B_{12}$ of 400 μg, 1.7 mg, and 2.4 μg, respectively, for nonpregnant, nonlactating individuals. Because a significant proportion of the population does not meet the current RDAs for folate intake, a reasonable population approach is to recommend an increase in the intake of foods containing those vitamins, ie, ready-to-eat fortified cereals, leafy green vegetables, fruits, and legumes as sources of folate; ready-to-eat fortified cereals, noncitrus fruits, poultry, beef, and certain vegetables (eg, artichoke, asparagus, beans, and cabbage) as sources of vitamin $B_6$; and beef, poultry, fish, and ready-to-eat fortified cereals as sources of vitamin $B_{12}$ (see Reference 11 for more extensive information). These dietary modifications could result in elevated vitamin status and perhaps decreased homocyst(e)ine levels. However, because 10% to 30% of older people may malabsorb food-bound $B_{12}$, it is advisable for those older than 50 years to meet their RDA mainly by consuming food fortified with $B_{12}$ or a $B_{12}$-containing supplement.

Moreover, on the basis of relative differences in bioavailability, the RDA guidelines equate 100 μg of folic acid from unfortified foods to 60 μg from fortified food and 50 μg from supplements. Because of methodological problems, food folate content in current databases may be significantly underestimated. Finally, prolonged heating or boiling, followed by discarding of water, or microwave heating may reduce folate content of food.

A complementary approach has been instituted as of January 1, 1998: the US Food and Drug Administration (FDA) has issued regulations requiring all "enriched" cereal grains to be fortified with folic acid at a concentration of 1.4 mg/kg grains to prevent neural tube birth defects. It has been estimated that this level of fortification would increase folic acid intake by 80 to 100 μg per day in women of childbearing potential and by 70 to 120 μg per day in adults older than 50 years. Whether this level of fortification will lower homocyst(e)ine concentrations in CAD patients needs to be determined. However, as described above, cereal products have been shown recently to lower homocyst(e)ine in CAD patients when they were fortified with 4 to 5 times the levels of fortification mandated by the US FDA.

## Detection and Management of Elevated Fasting Homocyst(e)ine

* * * *

Thus, some researchers consider that a reasonable approach is to determine levels of fasting homocyst(e)ine in "high-risk patients," ie, in those with strong family history for premature atherosclerosis or with arterial occlusive diseases, particularly in the absence of other risk factors, as well as in members for their families, because hyperhomocyst(e)inemia in CAD seems to be transmitted, at least in part, through an autosomal dominant mechanism. Other conditions that may be associated with high homocyst(e)ine are advanced age, hypothyroidism, impaired kidney function, systemic lupus erythematosus, and certain

medications, eg, nicotinic acid, nitrous oxide exposure, theophylline, methotrexate, and L-dopa.

After confirmation of high homocyst(e)ine concentration, it is important to check the vitamin status owing to the inverse relationships reported between homocyst(e)ine and blood levels of folate, B6, and B12. A useful algorithm for the diagnosis of vitamin B12 deficiency, beyond the determination of blood levels of this vitamin, is described in Reference 11.

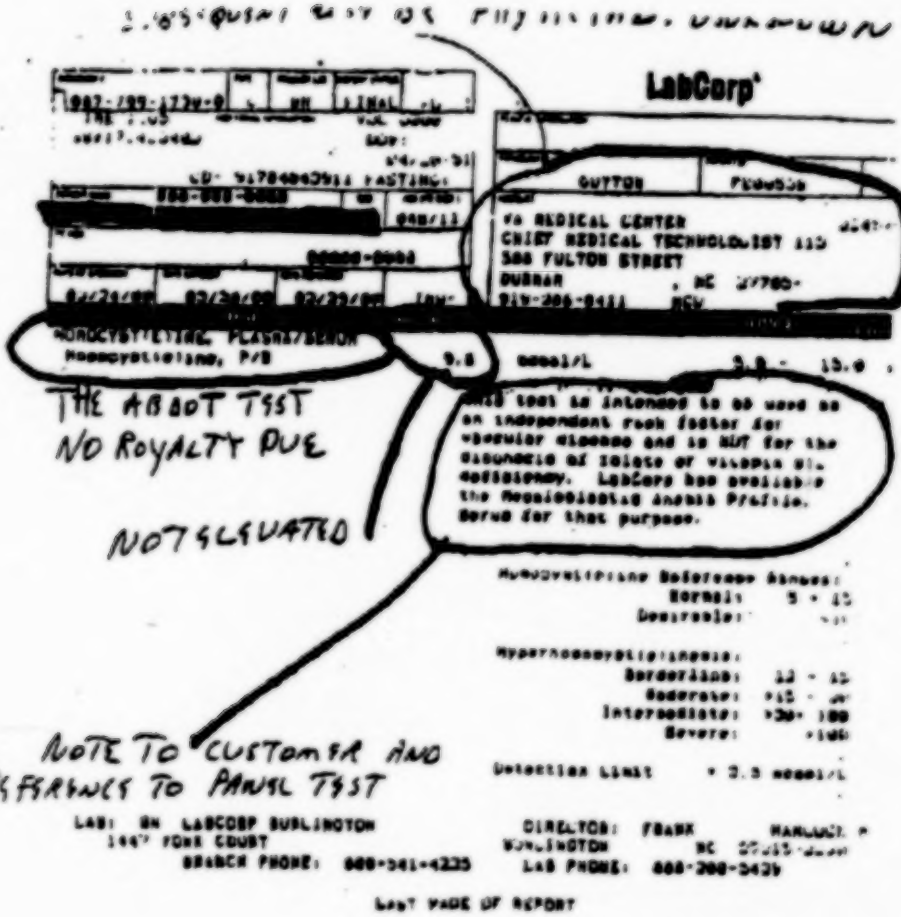
* * * *

**AHA Nutrition Committee January 5/12, 1999**

BEST AVAILABLE COPY



## Defendant's Exhibit LT




CONFIDENTIAL

L013481

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF COLORADO

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Civil Action. No. 99-Z-870

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METABOLITE LABORATORIES, INC.,
a Colorado corporation, and
COMPETITIVE TECHNOLOGIES, INC.,
a Delaware corporation,

Plaintiffs,

vs.

LABORATORY CORPORATION OF AMERICA
HOLDINGS d/b/a LABCORP,
a Delaware corporation,

Defendant.

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**DECLARATION OF PETER WENTZ**

I, Peter Wentz, declare and state as follows:

1. I am a resident of Elon College, North Carolina and have lived in Elon College since 1989. The following statements are based upon my own personal knowledge.

2. I am currently employed as Director of Toxicology for Defendant Laboratory Corporation of America Holdings, Inc. ("LabCorp") in Burlington, North Carolina. I have been employed by LabCorp (and its predecessor Roche Biomedical Laboratories, Inc.) at its Burlington lab since 1989. In my position as Director of Toxicology, I supervise approximately 45 LabCorp employees. I am responsible for ensuring that about 150 different tests conducted in the toxicology department are properly performed, including the tests licensed by Plaintiffs. LabCorp performs more than 2,000 different clinical tests. As a

clinical reference laboratory, LabCorp performs tests and provides results which assist physicians in diagnosing and treating diseases in their patients.

3. Prior to about August 1, 1998, LabCorp performed the single assay of homocysteine sold under test no. 706994 using the gas chromatography mass spectrometry method licensed to LabCorp by Metabolite. Beginning on or about August 1, 1998, LabCorp began performing the single assay of homocysteine sold under test no. 706994 using an immunoassay kit from Abbott Laboratories. Attached as Exhibit 1 hereto is a true and correct copy of a package insert from Abbott that describes its immunoassay method. This Abbott method does not use gas chromatography or mass spectrometry. Additionally, this Abbott method does not employ the steps recited in Claim 1 of the '658 patent of combining a sample containing homocysteine with an internal reference standard labelled with a suitable marker, measuring the relative amounts of labelled and unlabelled homocysteine with a mass spectrometer, or calculating the ratio of labelled and unlabelled homocysteine.

4. In some cases, LabCorp has included an assay of homocysteine under test no. 706994 in panels or superpanels containing other tests. For panels or superpanels performed after about July 31, 1998, which contained a homocysteine test no. 706994, those homocysteine tests were performed using the Abbott immunoassay method. None of the homocysteine tests (test no. 706994) included in panels or superpanels after about July 31, 1998 were performed using Metabolite's gas chromatography/mass spectrometry method.

5. Attached as Exhibit 2 hereto are copies of patient reports transmitting the results of assays of total homocysteine. In the Spring of 1999, language was included on LabCorp's homocysteine (test no. 706994) patient report to refer physicians to LabCorp's Megaloblastic Anemia profile test no. 706960 if the physicians were trying to diagnose a deficiency in cobalamin or folate.

This language is consistent with language that I proposed to Dr. Allen in 1992 when we first considered offering a single homocysteine test as a marker for cardiovascular disease.

6. Attached as Exhibit 3 hereto is a copy of the current Directory of Services page containing information on the single homocysteine test no. 706994. The information on that test refers the physician to LabCorp's Megaloblastic Anemia profile test (test no. 706960) to diagnose folate deficiency. LabCorp has paid and continues to pay royalties to plaintiffs on test no. 706960. The Megaloblastic Anemia profile test includes assays of four metabolites, methylmalonic acid. homocysteine, 2-methylcitric acid, and cystathionine. From the beginning, LabCorp has used the gas chromatography/mass spectrometry method licensed from Metabolite to assay each of the four metabolites, including homocysteine, for the Megaloblastic Anemia profile test. LabCorp continues to use the licensed gas chromatography/mass spectrometry method to assay each of the four metabolites, including homocysteine, for the Megaloblastic Anemia profile test.

7. LabCorp's Directory of Services contains a list of many of the more than 2,000 tests that LabCorp performs, including a description of each test to assist the physician in ordering tests. LabCorp listed the Megaloblastic Anemia profile test in its Directory of Services beginning in 1993 to inform physicians of the availability of the licensed test. Listing this test in LabCorp's Directory of Services would seem to be a benefit to Metabolite because LabCorp was paying Metabolite 27.5 percent of the revenue that LabCorp billed for each licensed test. Under that Agreement, Metabolite keeps 21.5 percent and pays 6.0 percent to CTI. LabCorp continues to pay a 27.5 percent royalty to Metabolite for each Megaloblastic Anemia profile test (test no. 706960) and other licensed tests performed by LabCorp that use Metabolite's gas chromatography/mass spectrometry method.

I declare under penalty of perjury of the laws of the United States of America that the foregoing is true and correct.

This the 2 day of August, 2000.

/s/ Peter Wentz

Peter Wentz

## JURY INSTRUCTIONS

### INSTRUCTION NO. 1

Now that you have heard the evidence, and the arguments of counsel, it becomes my duty to give you the instructions of the Court as to the law applicable to this case.

It is your duty as jurors to follow the law stated in the instructions of the Court and to apply the rules of law so given to the facts as you find them from the evidence in the case.

You are not to single out one instruction alone as stating the law, but must consider the instructions as a whole.

Neither are you to be concerned with the wisdom of any rule of law stated by the Court. Regardless of any opinion you may have as to what the law ought to be, it would be a violation of your sworn duty to base a verdict upon any other view of the law than that given in the instructions of the Court; just as it would be a violation of your sworn duty, as judges of the facts, to base a verdict upon anything but the evidence in this case.

Nothing which is said in these instructions is to be taken as an indication that the Court might have an opinion about the facts in the case, or what that opinion is. It is not the Court's function to determine the facts, but rather yours.

Justice through trial by jury depends upon the willingness of each individual juror to seek the truth as to the facts from the same evidence presented to all jurors; and to arrive at a fair verdict applying the same rules of law, as given in the instructions of the Court.

This case should be considered and decided by you as an action between persons of equal standing in the community, of equal worth, and holding the same or similar stations of life. A corporation is entitled to the same fair trial at your

hands as a private individual. All persons, including corporations, stand equal before the law, and are to be dealt with as equals in a court of justice.

## INSTRUCTION NO. 2

I will now explain the claims and defenses of each party to the case and the law governing the case. Please pay close attention to these instructions. These instructions include both general instructions and instructions specific to the claims and defenses in this case. You must consider all the general and specific instructions together. You must all agree on your verdict, applying the law , as you are now instructed, to the facts as you find them to be.

**The parties agree on the following facts.** The Professors assigned their rights to plaintiff CTI (then called University Patents, Inc.). The Patent Office issued the '658 patent to CTI. CTI licensed the patent to plaintiff Metabolite. In turn, Metabolite sublicensed the patent to a company called Roche, which became defendant LabCorp through a merger. Therefore, there is now a sublicense agreement between Metabolite and LabCorp. Even though this is technically a sublicense agreement, it is titled "License Agreement."

Next I will read to you contentions of the parties on which they do not agree.

**Plaintiffs make the following contentions**: The license agreement requires LabCorp to pay royalties amounting to 27.5% on its performance of any homocysteine tests if LabCorp's actions in performing such tests would, but for the existence of the License Agreement, infringe any valid claim of the patent either contributory or by inducement. The license agreement contains Section 4.02 addressing termination by LabCorp with respect to particular tests. Under that Section 4.02, LabCorp may terminate the license agreement with respect to homocysteine tests if it finds a more cost-effective commercial alternative and its performance of that

alternative does not infringe a valid and enforceable claim of the patent. The patent is presumed valid.

LabCorp decided sometime before 1998 that it might avoid its royalty obligations under the license agreement by switching to a homocysteine test sold by a company called Abbott. A LabCorp employee named Dr. Wentz made the determination that LabCorp's performance of homocysteine tests using the Abbott system did not infringe a valid and enforceable claim of the Professor's patent because the Abbott system is not by GCMS. On that basis, LabCorp terminated the license agreement with respect to the homocysteine-only test and stopped paying royalties on that test.

However, Dr. Wentz by his own admission is not competent to interpret patents, and knows nothing about the concepts of contributory infringement and infringement by inducement. In point of fact, LabCorp's performance of homocysteine-only tests using the Abbott System does constitute contributory infringement and infringement because claim 13 is not limited to GCMS and there are no substantial uses of the homocysteine-only tests other than to detect $B_{12}$ or folate deficiencies. Further, LabCorp induces infringement of claim 13 by encouraging physicians to assay for total homocysteine and to correlate the results with $B_{12}$ or folate deficiencies.

Therefore, LabCorp's termination of the license agreement and discontinuance of its royalty payments with respect to that test constitutes breach of its contract with Metabolite. And LabCorp's ongoing performance of these tests following its termination of the license agreement with respect to those tests, constitutes contributory infringement and infringement by inducement of the patent owned by CTI.

Monitoring patients for vascular disease is not a use of homocysteine testing separate from detecting vitamin deficiencies; this is evident from the fact that all parties agree that the standard treatment for elevated homocysteine in heart

disease patients is the same as the standard treatment for all other patent. The standard treatment is vitamin supplements.

**Defendant makes the following contentions**: Defendant LabCorp admits that it is licensed under the '658 patent by Metabolite and does not have any liability for patent infringement. LabCorp denies that it owes plaintiffs any additional royalties under the license agreement. LabCorp did not terminate the license agreement or any portion of it with respect to homocysteine tests because LabCorp continues to perform certain homocysteine tests by the licensed gas chromatography/mass spectrometry method and continues to pay royalties on those homocysteine tests to plaintiffs. Defendant does not owe Metabolite a 21.5% royalty for performing the Abbott single homocysteine test because the assay is not a Licensed Assay as defined by the Licensed Assay and does not use Metabolite's technology.

LabCorp began using the Abbott method to perform certain single homocysteine test in August 1998 because it was quicker and more cost effective. Section 4.02 of the license agreement recognizes that LabCorp is entitled to use more cost-effective methods to perform assays for homocysteine. Dr. Wentz was correct in determining that LabCorp's performance of the single homocysteine test by the Abbott method is not covered by claim 13 of the '658 patent because the Court has specifically entered an order stating that LabCorp's performance of the single homocysteine test by the Abbott method is not covered by claim 13. After assaying a blood sample for homocysteine, LabCorp does not take any further action to correlate an elevated level, if any, with a vitamin deficiency but simply reports the result. In fact, over 80% of the results are not elevated. Although Dr. Wentz is not a patent lawyer, he was correct on this issue. On the other hand, plaintiffs CTI and Metabolite, who have been represented by patent attorneys, incorrectly accused LabCorp of directly infringing claim 13 of the '658 patent.

LabCorp has not induced or contributed to the infringement of any claim of the '658 patent because the single homocysteine tests have a substantial noninfringing use, including to screen and monitor patients for risk of heart disease. The American Heart Association has specifically recommended this use of the single homocysteine assays based on a discovery made by Dr. Kilmer McCully in 1969. CTI admitted this use of the assays in a 1999 press release.

Defendant contends that claims 13 and 18 are invalid and unenforceable for several reasons.

**INSTRUCTION NO. 3**

Each party in this case asserts that certain facts exist in support of its claim or its defense and each party must prove such facts by the required weight of evidence known as the burden of proof. Each party's burden of proof remains the same throughout the case, *i.e.*, it never changes.

In human affairs, and even in scientific matters, absolute certainty of facts is seldom possible. Thus, proof of facts to an absolute certainty is not required. To protect the interests of the parties and to foster certain public policy goals, the law imposes different burdens in different types of cases. For instance, in criminal cases the defendant's guilt has to be proven "beyond a reasonable doubt." In civil cases, like this one, lower standards or burdens of proof are used.

Generally speaking, the burden of proof in civil cases, like this one, is known as the "preponderance of evidence" standard. Sometimes the law imposes a heavier burden of proof known as the "clear and convincing" evidence standard because of other public policy considerations. I will tell you when to apply each burden of proof standard.

There will be two burden of proof standards by which you must weigh the evidence in this case. Plaintiff has the burden of establishing infringement of the claims of its patent by a preponderance of the evidence. Defendant has the burden of

establishing patent invalidity and/or unenforceability by the heavier clear and convincing evidence standard.

I will instruct you as to the standard by which you must weigh the evidence on each issue involved in this case. In determining whether any fact in issue has been proven under either standard, you should consider the testimony of all witnesses, regardless of who called them, and a exhibits, regardless of who offered them, including all reasonable inferences from the testimony and exhibits as seem justified in view of your experience and common sense.

A preponderance of the evidence means such evidence which, when considered and compared with that opposed to it, has more convincing force and produces in your minds a belief that what is sought to be proved is more likely true than not true. Put in a slightly different way, a party's burden to establish or prove a claim by a preponderance of the evidence means proof which produces in your minds the belief that the facts offered in support of the claims are more likely so than not so.

Clear and convincing evidence involves a greater degree of persuasion than that which will satisfy a preponderance of the evidence standard. The evidence must be highly probable and free from serious doubt. For purposes of evaluating evidence where a party has the burden of proof by clear and convincing evidence, that evidence must produce in your minds a firm belief or conviction as to the matter sought to be established. You must be convinced that the fact which that party seeks to prove is indeed true in order to meet the clear and convincing burden.

## INSTRUCTION NO. 4

The evidence in the case consists of all the sworn testimony of all the witnesses, all exhibits which have been received in evidence, all facts which have been admitted or agreed to.

In deciding the facts, you must consider only the evidence received at trial. Evidence offered at the trial and rejected or stricken by the Court must not be considered by you. Statements, remarks, arguments, and objections by counsel and remarks of the Court not directed to you are not evidence.

You are to consider only the evidence in the case and the reasonable inferences from that evidence. An inference is a decision or conclusion which reason and common sense lead the jury to draw from other facts which have been proved.

## INSTRUCTION NO. 5

Any finding of fact you make must be based on probabilities, not possibilities. You should not guess or speculate about a fact.

## INSTRUCTION NO. 6

Evidence may be either direct or circumstantial. Circumstantial evidence is the proof of facts or circumstances from which the existence or nonexistence of other facts may reasonably be inferred. All other evidence is direct evidence. The law makes no distinction between the effect of direct evidence and circumstantial evidence.

## INSTRUCTION NO. 7

The weight of evidence is not necessarily determined by the number of witnesses testifying to a particular fact.

## INSTRUCTION NO. 8

The lawyers have highlighted certain parts of some exhibits. However, it is for you to determine the significance of the highlighted parts.

## INSTRUCTION NO. 9

When a corporation is involved, of course, it may act only through natural persons as its agents or employees; and, in general, any agent or employee of a corporation may bind the corporation by his acts and declarations made while acting within the scope of his authority delegated to him by the corporation, or within the scope of his duties as an employee of the corporation.

## INSTRUCTION NO. 10

You are the sole judges of the credibility of the witnesses and the weight to be given their testimony. You should take into consideration their means of knowledge; strength of memory and opportunities for observation; the reasonableness or unreasonableness of their testimony; the consistency or lack of consistency in their testimony; their motives; whether their testimony has been contradicted or supported by other evidence; their bias, prejudice or interest, if any; their manner or demeanor upon the witness stand; and all other facts and circumstances shown by the evidence which affect the credibility of the witnesses.

Based on these considerations, you may believe all, part or none of the testimony of a witness.

## INSTRUCTION NO. 11

A witness qualified as an expert by education, training, or experience may state opinions. You should judge expert testimony just as you would judge any other testimony. You may accept it or reject it, in whole or in part. You should give the testimony the importance you think it deserves,

considering the witness's qualifications, the reasons for the opinions, and all of the other evidence in the case.

**INSTRUCTION NO. 12**

Certain testimony has been read into evidence or presented as videotape evidence from a deposition. A deposition is testimony taken under oath before the trial and preserved in writing. You are to consider that testimony as if it had been given by the witness from the witness stand.

**INSTRUCTION NO. 13**

Plaintiff Metabolite's first claim against defendant is for breach of the licensing agreement or contract. To establish their claim against the defendant, plaintiffs must prove that:

1. Metabolite did what the contract required Metabolite to do.

2. Defendant did not do what the contract required defendant to do. This is called a breach of the contract.

3. Defendant's breach, or failure to do what the contract required, caused a loss to plaintiffs.

If you find plaintiffs proved these three elements, then your verdict must be for plaintiffs on this claim. If you find that plaintiffs have not proved these elements, then your verdict must be for defendant on this claim.

**INSTRUCTION NO. 14**

A. Plaintiffs claim the following terms were part of the license agreement.

(1) LabCorp was required to pay plaintiffs a royalty of 27.5% of the billed amount on each homocysteine test and each methylmalonic acid test within 60 days after each calendar quarter.

(2) LabCorp was required to include with each royalty report to plaintiffs a certification by an officer certifying that the royalty report was accurate.

(3) LabCorp was prohibited from terminating the license agreement with respect to any particular homocysteine test unless the test was replaced by another test and LabCorp's actions in performing the other test did not infringe any valid and enforceable claim of the patent, either directly, contributorily or by inducement.

(4) LabCorp was required to give plaintiffs 60 days advance notice of any termination of the agreement with respect to any homocysteine or methylmalonic acid test.

B. Defendant claims the following terms were part of the license agreement.

"(1) LabCorp is only required to pay a royalty of 21.5% of net sales actually received on Licensed Assays to Metabolite and a royalty of 6% of net revenue billed after discounts and adjustments on Licensed Assays to Competitive Technologies. Payments are due within 60 days after each calendar quarter.

(2) LabCorp is entitled to perform the single homocysteine assay by the Abbott method which is not covered by the license agreement or the '658 patent without providing notice or terminating the agreement because LabCorp continues to use the licensed gas chromatography mass spectrometry method for assaying homocysteine in the panel test.

Plaintiffs have the burden to prove what the parties intended the contract to mean. The contract is to be interpreted so as to give effect to the parties' intentions. You cannot make for the parties a better contract than the parties made for themselves. It is the intent expressed or apparent in the writing that controls.

The interpretive principles which follow are not intended to be exhaustive, but they are intended to cover the most frequently utilized principles.

In deciding what the parties intended, you may consider the relations of the parties, the attendant circumstances and the result the parties sought to attain.

A supporting or less significant provision of the contract is not to be interpreted to conflict with an obvious, dominant or principal purpose of the contract.

You should carefully consider the wording that was used in the contract. The terms of a contract, generally, are to be understood in their plain ordinary sense. The contract is to be considered as a whole and its provisions are to be read together. The conduct of the parties, however, after they entered into the contract and before they discovered that they disagreed with one another, can be significant evidence of their agreed intent. It is up to you to decide what the conduct of the parties was, whether the conduct is reasonably related to the terms in question and whether it reveals what they intended by the contract.

A course of dealing is the manner by which parties to the contract have previously dealt with each other. Such a course of previous dealing, can, unless specifically rejected in the contract, fairly be regarding as establishing a basis for interpreting and giving meaning to the parties' intention as it relates to this contract.

If any contract words or terms have a technical meaning, or as used in a trade or by custom mean something different from their ordinary meaning, you shall give them their technical trade or custom meaning if (1) the contract was made in view of this technical meaning, trade or custom usage, and (2) the technical meaning or trade or custom usage was either generally used or was actually known to the parties.

If you have considered all of the evidence to ascertain the intentions of the parties and you are still unable to decide what the parties originally intended the disputed contract language to mean, then that language as it exists should be interpreted against the party to who wrote the contract. Although the general rule is that ambiguity in a contract provision should be resolved against the drafter, the ambiguous provision must be read sensibly and consistent with the expressed intent of the parties.

**INSTRUCTION NO. 15**

The second element of the breach of contract claim is that the contract was breached. Failure to perform a contract in accordance with its terms constitutes a breach of contract. It does not matter if the failure was purposeful or inadvertent.

A. Plaintiffs claim defendant breached the license agreement in the following manner:

(1) Defendant wrongfully terminated the license agreement with respect to the homocysteine-only test. This is because defendant's actions in performing that test constitute, but for the existence of the license agreement, infringement of the patent either directly, contributorily or by inducement.

(2) Defendant failed to comply with the provision of the License Agreement requiring 60 days advance notice to Metabolite of a termination with respect to any particular test.

B. Defendant denies this. Defendant contends:

(1) LabCorp did not terminate the License Agreement with respect to any Licensed Assay. LabCorp continues to perform homocysteine assays by gas chromotography/mass spectrometry, and pays plaintiffs royalties on those assays.

(2) LabCorp does not owe any royalties for the single homocysteine test performed by the Abbott method because it is not covered by the license agreement or the '658 patent.

## INSTRUCTION NO. 16

A plaintiff - Metabolite in this case - who is awarded a verdict for breach of contract is entitled to compensatory damages for such losses as may fairly be considered to have arisen naturally from defendants' breach of contract. Alternatively, Metabolite may be entitled to such damages as may reasonably be supposed to have been contemplated by both parties, at the time they made the contract, as the probable result of the breach of such contract.

Compensatory damages for breach of contract are designed under the law to place the injured party in as good a monetary position as it would have enjoyed if the contract had been performed as promised.

## INSTRUCTION NO. 17

Now, I will turn to the patent issues. I will be instructing you on issues where it will be necessary for you to determine what is a person of ordinary skill in the field of homocysteine and vitamins $B_{12}$ and folate at the time the invention was made. This is called a person of ordinary skill in the art.

LabCorp contends that claims 13 and 18 of the '658 patent are invalid because the claimed invention was obvious to one of ordinary skill in the art at the time the invention was made. LabCorp bears the burden of proving this defense by clear and convincing evidence.

A person of ordinary skill in the art is presumed to be one who thinks along the line of conventional wisdom in the art. He or she is not one who undertakes to innovate, either by patient and expensive systematic research or by extraordinary insights.

The parties agree that the level of ordinary skill in the field of medicine relating to homocysteine was a person having a medical degree and experience in researching the amino acid, homocysteine, and its relationship to diseases.

When you decide the issue of obviousness, you must decide whether or not the invention would have been obvious to one having this ordinary level of skill in the field of science and medicine relating to homocysteine.

To be patentable an invention must not have been obvious to a person of ordinary skill in the pertinent art at the time the invention was made. The issue is not whether the claimed invention would have been obvious to you as a layman, to me as a judge, or to a genius in the art, but whether it would have been obvious to one of ordinary skill in the field of science and medicine of homocysteine at the time it was made.

You should conclude that the patent in suit is invalid even though not identically disclosed in a single prior art device of reference, only if LabCorp has established by clear and convincing evidence facts which show that the claimed invention as a whole would have been obvious to a person of ordinary skill in the field of science and medicine of homocysteine at the time the invention was made.

Before determining whether or not LabCorp has established obviousness of the claimed invention, you must determine the following factual matters, each of which must be established by clear and convincing evidence:

1. The scope and content of the prior art relied upon by LabCorp;

2. The difference or differences, if any, between each claim of the '658 patent and the prior art; and

3. The level of ordinary skill in the art at the time the invention of the '658 patent was made.

4. Commercial success; long-felt need; independent invention by others; failure by others; copying; acceptance of licenses.

Against this background of facts you will then make your conclusion whether or not the claimed subject matter would have been obvious to a person of ordinary skill in the art of medicine relating to the use of assays of homocysteine at the time the invention was made. When doing so, each claim must be considered in its entirety and separately from the other claims.

**INSTRUCTION NO. 18**

I will now instruct you as to the rules you are to follow in arriving at your decisions as to whether defendant has infringed claim 13 of the '658 patent owned by plaintiff Competitive Technologies by inducement and contributorily. The Court has held that LabCorp does not directly infringe claim 13. However, I have not determined, and leave for your determination whether LabCorp's performance of its homocysteine-only test constitutes contributory infringement or infringement by inducement of claim 13. You will be asked to answer specific questions in the verdict form about these decisions.

In determining infringement, you must consider the scope of the claims as I have interpreted them. Although you have heard discussions during the trial as to embodiments or examples in the '658 description, a claim is not necessarily limited to such embodiments or specific examples in the patent. The law does not require the impossible and does not require that the applicant describe in his specification every conceivable and possible future embodiment of his invention. The specification merely illustrates at least one embodiment of the invention and teaches a person of ordinary skill in the art how to make and use it. The scope of the patent grant is not limited to this embodiment, but is limited only by what is within the scope of the claims.

I will now read you claim 13 as construed by the Court.

Claim 13: A method for "determining the existence of" "a shortage of cobalamin or folate necessary to health" in warm-

blooded animals comprising the steps of 1) assaying a body fluid for a level of total homocysteine "raised above the normal range" and 2) to establish a mutual or reciprocal relationship between" a level of total homocysteine "raised above the normal range" in said body fluid with "a shortage of cobalamin or folate necessary to health." the latter step describes a discrete step in a sequential process.

LabCorp cannot avoid infringement by the mere fact that the accused homocysteine-only test may be more or less efficient or may include features or functions not found in the patent.

**INSTRUCTION NO. 19**

You will note that there is a part of plaintiffs claim which reads: ". . . comprising . . .". Such a word means that the claim is open-ended. This means that if you find that a method contains each and every one of the elements of any of the patent claims but, in addition, also contains additional steps, then acts in connection with its performance of its homocysteine-only test nevertheless infringes.

**INSTRUCTION NO. 20**

In this case, plaintiffs assert that defendant has contributorily or by inducement infringed claim 13 of the '658 patent. Infringement occurs if you find that the plaintiffs have proven by a preponderance of the evidence that others have performed the second step of claim 13 using assays done by defendant and that defendant has contributed to or induced that infringement by others. It is not necessary for the infringer to be made a party to a case against one who infringes by inducement or contributorily. I will later instruct you on contributory and inducing infringement.

Infringement does not depend on bad faith. A person can infringe a patent even though in good faith it believes that what it is doing is not an infringement of the patent. It may also infringe even though in good faith it believes that what it is doing is not an infringement of any patent.

## INSTRUCTION NO. 21

To show infringement of claim 13 by inducement, plaintiffs must prove by a preponderance of the evidence that someone has directly infringed claim 13. If there is no direct infringement of claim 13 by anyone, defendant has not induced infringement. If you find that someone has directly infringed claim 13, it is not necessary to show that defendant LabCorp itself has directly infringed, if plaintiffs prove by a preponderance of the evidence that LabCorp actively and knowingly aided and abetted that direct infringement. Plaintiffs must show that LabCorp knew or should have known that its actions would induce actual infringement. LabCorp cannot be liable for inducing infringement if it had no reason to be aware of the existence of the patent.

If you find that someone has directly infringed claim 13 and that LabCorp knew or should have known that its actions would induce direct infringement, you may find that LabCorp induced another to infringe claim 13 if it provided instructions and directions to perform the infringing act through labels, advertising or other sales methods. You may find that LabCorp induced infringement even if there is an express warning against the infringement, if the material containing the warning nonetheless invites the infringing activities under the circumstances. You may find that LabCorp induced infringement by supplying the components that are used in an infringing method or apparatus with the knowledge and intent that its customer would directly infringe by using the components to make, use or sell the patented invention.

## INSTRUCTION NO. 22

Plaintiffs assert that defendant is liable for contributory infringement of claim 13. Plaintiffs has the burden to prove contributory infringement by a preponderance of the evidence. To establish contributory infringement by defendant, plaintiffs must first show that someone directly infringed the '658 patent; however, plaintiffs do not need to prove that

defendant itself directly infringed. If there is no direct infringement by anyone, there can be no contributory infringement.

If you find someone has directly infringed the patent, then contributory infringement exists if plaintiffs prove that:

(1) LabCorp sold or supplied;

(2) a material component of the patented invention that is not a staple article of commerce capable of substantial noninfringing use;

(3) with knowledge that the component was especially made or adapted for use in an infringing method.

A "staple article of commerce capable of substantial noninfringing use" is something that has uses other than the patented method and that those other uses are not occasional, farfetched, impractical, experimental or hypothetical.

**INSTRUCTION NO. 23**

Now I will instruct you about certain defenses defendant has raised to plaintiffs' allegations of patent infringement.

Under the law, plaintiff's patent is presumed valid. The presumption of validity goes to the validity of plaintiff's patent in relation to the patent statute as a whole, no matter on what grounds defendant seeks to invalidate plaintiff's '658 patent.

Each claim of plaintiff's patent (whether in independent, dependent, or multiple dependent form) shall be presumed valid independently of what you find as to the validity of other claims; dependent or multiple dependent claims shall be presumed valid even though dependent upon what you find to be an invalid claim.

The burden upon defendant, who challenges the validity of the '658 patent, is to introduce evidence of facts establishing

invalidity (thus overcoming the presumption of validity). That evidence, if defendant is to prevail, must be clear and convincing. That is a heavier burden than preponderance of the evidence.

Because you have heard that defendant has introduced prior art not considered by the examiner, a situation which is common in a patent trial, this does not "weaken" or otherwise affect the presumption of validity.

In the end, you must decide whether all the evidence introduced by both sides, whether considered by the examiner or not, establishes that defendant has carried its burden so as to have persuaded you by clear and convincing evidence that the '658 patent can no longer be accepted as valid.

**INSTRUCTION NO. 24**

If you find by a preponderance of the evidence that LabCorp was licensed under the '658 patent and that the license has not been terminated with respect to the tests at issue, then you must find that LabCorp has not infringed the '658 patent.

**INSTRUCTION NO. 25**

You have heard the term "prior art" used by the parties in this trial. These terms are used in connection with facts or events which by law prevent an applicant from obtaining a patent or, if a patent has been issued, renders such a patent invalid. The law provides that a patent cannot be obtained if the invention was:

1. known or used by others in this country before the date of invention by the inventors of the patent; or

2. patented or described in a printed publication in this or a foreign country more than one year prior to the date of the application which resulted in the patent.

I will now review these items with you separately with regard to the evidence you have heard in this case.

## INSTRUCTION NO. 26

In determining whether the invention of the patent in suit would have been obvious to a person of ordinary skill in the art, you must presume that person would have known about all of the relevant prior art. A combination of prior art cannot render an invention obvious unless every one of the elements of the claimed invention is disclosed in at least one of those items of prior art. In examining the prior publications, you should take into account not only what they expressly disclose, but also anything that inherently occurred as a result of practicing what was expressly disclosed in that prior art.

If you find that a combination of items of the prior art showed each of the elements of the claims in suit, you must determine whether or not it would then have been obvious to a person of ordinary skill in the art of medicine relating to homocysteine to combine or coordinate these elements in the same manner as the claims of the '658 patent. You must not use hindsight in answering this question. In other words, it is wrong to use the '658 patent as a guide through the prior art references, combining the right references in the right way so as to achieve the result of the claims of the '658 patent. Rather, you must determine whether or not, at the time the invention was made, the invention of the '658 patent would have been obvious to one of ordinary skill in the art having knowledge of all the prior art.

If you conclude that the prior art discloses all the elements of the claimed invention, but those elements are in separate prior art references, you must then consider whether or not it would have been obvious to combine the elements. To answer this question "yes," you must determine that there was some suggestion in the prior art to combine the elements. The suggestion can be expressly stated in a particular reference or it can be within the knowledge that was generally available to one of ordinary skill in the art of medicine relating to homocysteine.

## INSTRUCTION NO. 27

In deciding whether the prior art suggests the method recited in the claims of the '658 patent, you are instructed that if the prior art merely discloses numerous possible combinations but gives no direction as to which of those of many choices is likely to be successful, this does not constitute a suggestion of the claimed method. Similarly, if the prior art merely discloses that it would be obvious to explore a new technology or general approach that seemed to be a promising field of experimentation, this would not constitute a suggestion of the claimed method.

On the other hand, for the prior art to "suggest" the claimed invention, it is not necessary that one of ordinary skill in the medical art, looking at the prior art, could predict with certainty that the claimed invention would be successful. Rather, it is sufficient that a person of ordinary skill in the medical art, looking at the prior art, would have a reasonable expectation that the claimed invention would achieve the desired result.

## INSTRUCTION NO. 28

Before deciding the issue of obviousness, you must consider certain factors. Some weigh in favor of finding that the invention was obvious; some do not.

One of the factors you should consider is whether or not plaintiff has shown any commercial success of products covered by the patent in suit due to the merits of the claimed invention. To prove this, plaintiff would have to satisfy you that there is a causal connection between the evidence showing the commercial success and the claimed invention. For example, if you conclude that commercial success is due to advertising, promotion, salesmanship or the like, or to features of the product other than those claimed in the patent in suit, rather than to the claimed invention, then it is not established that commercial success has a relation to the invention itself. If, however, you were to find that the

invention of the '658 patent achieved commercial success and that the merits of the invention caused the commercial success this would tend to indicate that the invention was not obvious.

You should also consider whether or not the claimed invention was invented independently by other persons, either before it was invented by the inventor of the patent in suit or at about the same time. Just as the failure of others to make the invention may be evidence that an invention was not obvious, independent making of the invention by persons other than the inventor about the same time may be evidence that the invention was obvious.

**INSTRUCTION NO. 29**

Another factor you should consider is whether or not plaintiff has shown that others had tried, but failed to solve the problem solved by the invention of the '658 patent. To prove this plaintiff must show that others had tried and failed and that it was the merits of the claimed invention that allowed the inventors of the '658 patent to succeed where others had failed. For example, if advances in unrelated technology allowed the inventor of the '658 patent to succeed where others had failed, then plaintiff would not have carried his burden of proof on this issue. If, however, you were to find that the merits of the invention allowed the inventor to succeed where others had failed, this would tend to indicate that the invention was not obvious.

Another factor you should consider is whether or not plaintiff has shown copying by others of the invention claimed in the '658 patent. If you were to find that others copied the invention because of its merits this would tend to indicate that the invention was not obvious.

Another factor you should consider is whether or not plaintiff has shown unexpected superior results achieved by the invention claimed in the '658 patent. To prove this plaintiff must show that it was the claimed invention that

caused the unexpected results. For example, if there were not unexpected superior results or the unexpected results were due to a feature unrelated to the claimed invention, then plaintiff would not have carried its burden of proof on this issue. If, however, you were to find that there were unexpected superior results which were related to the claim invention, this would tend to indicate that the invention was not obvious.

Another factor you should consider is whether or not plaintiff has shown that others have accepted licenses under the '658 patent. To prove this plaintiff must show that others agreed to licenses because of the merits of the claimed invention. For example, if the others accepted licenses due to factors such as the cost of litigation, or the low cost of the license, then it has not been established that the acceptance of licenses was due to the merits of the invention. itself. If, however, you were to find that others took licenses as a result of the merits of the claimed invention, this would tend to indicate that the invention was not obvious.

**INSTRUCTION NO. 30**

One of the requirements for a valid patent is that the invention defined in the claims must be new. This is known as the requirement of novelty. Something that shows that an invention was not new or novel is called an anticipation. There are several categories of events, all defined by the patent laws, that can anticipate and invalidate a patent. In this case, LabCorp contends that the claim 13 is of the '658 patent is anticipated because the invention of claim 13 of the '658 patent was described in the Refsum et al. article published by "Clinical Chemistry" in Vol. 31, no. 4 (1985) which Applicants acknowledged was prior art.

I will now instruct you as to the rules of law that you must follow in deciding whether or not the claim 13 of the '658 patent was anticipated.

First, LabCorp has the burden of proving anticipation by clear and convincing evidence.

Second, a publication anticipates a claim if it discloses the claimed invention sufficiently that a person of ordinary skill in the art could take its teachings together with his own knowledge of the art and be in possession of the claimed invention. To establish that anticipation exists, LabCorp must show that each of the elements recited in the claim was disclosed in the particular item claimed to be an anticipation. If every element of the claimed invention is shown to be present in the prior publication, the patent claim is anticipated.

Third, there is no anticipation unless every one of the elements of the claimed invention is found in a single prior publication. You may not combine two or more items of prior art to make out an anticipation. You should, however, take into consideration not only what is expressly disclosed or embodied in the particular item of prior publication but also what inherently occurred as a natural result of its practice. A party claiming inherency must prove it by clear and convincing evidence. It must show that the publication always inherently produces a process meeting all of the claim limitations. In determining whether or not every one of the elements of the claimed invention is found in the prior publication you should take into account what a person of ordinary skill in the art would have understood from his or her examination of the particular publication.

You must keep these requirements in mind and apply them to each kind of anticipation you consider in this case. There are additional requirements that apply to the particular categories of anticipation that LabCorp contends apply in this case. I will now instruct you about those.

**INSTRUCTION NO. 31**

The requirements of patentability require that the specification conclude with one or more claims that particularly

pointing out and distinctly claim the subject matter which the Applicant regards as his invention. Each claim must be considered separately.

If you find by clear and convincing evidence that claim 13 or claim 18 does not particularly point out and distinctly claim the invention, then you may find the claim is invalid for indefiniteness.

**INSTRUCTION NO. 32**

LabCorp contends that the Applicants (CTI and Allen) engaged in inequitable conduct before the United States Patent and Trademark Office. The question of whether Applicants engaged in inequitable conduct is a question for me to decide. However, I will decide that question based upon whether you find the following facts have been proved by clear and convincing evidence:

1. The Applicants, CTI or Allen, made a misrepresentation to the Patent and Trademark Office;

2. That any such misrepresentation was material; and

3. The degree to which you find that the Applicants acted with intent.

Applicants have a duty of honesty and candor when dealing with the patent office which precludes them making intentional, material misrepresentations. Misrepresentations can be in the form of either a false statement of a material fact or the omission of a material fact from a statement that was made. A fact is material when there is a substantial likelihood that a reasonable patent examiner would consider it important in deciding whether to allow the application as a patent.

The question of intent may be established by circumstantial evidence, based upon a person's outward manifestations, the person's words, conduct, acts, and all the surrounding circumstances disclosed by the evidence together with the

rational and logical inferences that may be drawn from them. If you find that CTI or Allen knowingly submitted a false or misleading statement that was material to the consideration of the patent application, you may, but are not required to, conclude that the statement was part of a deliberate scheme to deceive the Patent and Trademark Office.

Based on your findings of whether the misrepresentation was made, its materiality, and the intent of CTI or Allen, I will make the determination of whether CTI or Allen engaged in inequitable conduct.

**INSTRUCTION NO. 33**

Under the U.S. patent laws, a patent must disclose sufficient information to enable those skilled in the art to make and use the claimed invention. This requirement is known as the enablement requirement.

The defendant in this case has asserted that the plaintiff's patent is invalid for failure to satisfy the enablement requirement. As with assertions of patent invalidity on other grounds, the defendant bears the burden of establishing nonenablement by clear and convincing evidence.

To determine whether a patent is enabling, one must look to the time the application for that patent was filed. A patent is enabling if its disclosure is sufficient to enable a person of ordinary skill in the art to make and use the claimed invention. A patent need not disclose what is well known in the art.

A patent is enabling even if some experimentation would be required to achieve the claimed invention, so long as that amount of experimentation is routine and would not be considered undue by those skilled in the art. Factors to be considered in determining whether a disclosure would require undue experimentation include: (1) the quantity of experimentation necessary; (2) the amount of direction or guidance disclosed in the patent; (3) the presence or absence of working examples in the patent; (4) the nature of the

invention; (5) the state of the prior art; (6) the relative skill of those in the art; (7) the predictability of the art; and (8) the breadth of the claims.

## INSTRUCTION NO. 34

I will now instruct you on the law you must follow in determining patent infringement damages, if any.

The patent law specifically provides that the amount of damages that defendant must pay plaintiff for infringing plaintiff CTI's patent may not be less than a reasonable royalty for the use that defendant made of plaintiff's invention.

## INSTRUCTION NO. 35

A royalty is a payment made to the owner of a patent by a non-owner in exchange for rights to make, use or sell the claimed invention. A reasonable royalty is the royalty that would have resulted from a hypothetical negotiation between plaintiff and a company in the position of defendant taking place at the time that the infringement began. You should also assume that both parties to that negotiation understood the patent to be valid and infringed and that the licensee would respect the patent.

## INSTRUCTION NO. 36

In determining the value of a reasonable royalty, you may consider evidence on any of the following factors.

1. Any royalties received by plaintiff for the licensing of the patent in suit, proving or tending to prove an established royalty.

2. The rates paid by defendant to license other patents comparable to the '658 patent.

3. Plaintiff's established policy and marketing program to maintain his right to exclude others from using the patented invention by not licensing others to use the invention, or by

granting licenses under special conditions designed to preserve that exclusivity.

4. The commercial relationship between the licensor and the licensee, such as whether or not they are competitors in the same territory in the same line of business.

5. The effect of selling the patented product in promoting sales of other products of the licensee; the editing value of the invention to the licensor as a generator of sales of his non-patented items; and the extent of such collateral sales.

6. The duration of the patent.

7. The established profitability of the product made under the patent; its commercial success; and its current popularity.

8. The utility and advantages of the patented invention over the old modes or devices, if any, that had been used for achieving similar results.

9. The nature of the patented invention; the character of the commercial embodiment of it as owned and produced by the licensor; and the benefits to those who have used the invention.

10. The extent to which the accused infringer has made use of the invention; and any evidence that shows the value of that use.

11. The portion of the profit or of the selling price that may be customary in the particular business or in comparable businesses to allow for the use of the invention or analogous inventions.

12. The portion of the profit that arises from the patented invention itself as opposed to profit arising from features unrelated to the patented invention, such as the manufacturing process, business risks, or significant features or improvements added by the accused infringer.

## INSTRUCTION NO. 37

Although the relevant date for the hypothetical reasonable royalty negotiation is the date that the infringement began, you may consider in your determination of reasonable royalty damages any actual profits by the defendant after that time and any commercial success of the patented invention in the form of sales of the patented or infringing products after that time. You may only consider this information, however, if it was foreseeable at the time that the infringement began.

## INSTRUCTION NO. 38

You must not award plaintiff CTI more than the amount of damages adequate to compensate plaintiff CTI for defendant's infringement. You must not include any additional amount for the purpose of punishing defendant or setting an example.

In determining damages, you must not consider plaintiff's claims of willfulness, or take into account any evidence relating to those claims. The consideration of willfulness, if any, is entirely separate from the question of any damages you are asked to determine.

You should not increase damages because you find willfulness, or decrease damages because you do not find willfulness. In addition, you should not include any amount in your damages award for interest, attorney fees, or other expenses.

## INSTRUCTION NO. 39

The patent owner has the burden of proving each element of its damages by a preponderance of the evidence.

## INSTRUCTION NO. 40

You are instructed that you should begin calculating patent infringement damages as of the date that the infringement began on August 1, 1998.

## INSTRUCTION NO. 41

You are instructed that where a potential infringer has actual notice of another's patent rights, he has an affirmative duty of due care not to infringe. Thus if you find by a preponderance of the evidence that LabCorp infringed the patent, then you must further determine if this infringement was willful. Willfulness must be proven by clear and convincing evidence showing that:

A. LabCorp had actual knowledge of the patent, and

B. LabCorp had no reasonable basis for believing that (a) LabCorp did not infringe the patent or (b) that the patent was invalid.

In making the determination as to willfulness, you must consider the totality of the circumstances. The totality of the circumstances comprises a number of factors, which include, but are not limited to whether the defendant intentionally copied the product covered by the patent, whether the defendant exercised due case to avoid infringing the patent, whether the defendant relied on competent legal advice, and the defendant's behavior as a party to the litigation.

## INSTRUCTION NO. 42

You may not include damages that are speculative, only possible, or that are based on guesswork. Difficulty or uncertainty in determining the precise amount of any damages does not prevent you from deciding an amount. You should use your best judgment based on the evidence.

## INSTRUCTION NO. 43

The fact that I have instructed you on the proper measure of damages should not be considered as any suggestion on which party is entitled to your verdict in this case. Instructions on the measure of damages are given for your guidance, in the event you find in favor of the plaintiff from a preponderance of the evidence in the case in accordance with the other instructions

## INSTRUCTION NO. 44

These instructions contain the law that you must use in deciding this case. No single instruction states all the applicable law. All the instructions must be read and considered together.

You must not be concerned with the wisdom of any rule of law. Regardless of any opinion you may have as to what the law should be, it would be a violation of your sworn duty to base a verdict upon any other view of the law than that given in the instructions of the Court.

I do not, by these instructions, express any opinions as to what has or has not been proved in the case, or to what are or are not the facts of the case.

## INSTRUCTION NO. 45

The Marshal will now escort you to the jury room. After you get to the jury room you shall select one of your members to be the foreperson of the jury. That persons will be in charge of your discussions. You must all agree on your verdict, and the foreperson must sign and date the original form of whatever verdict you reach.

(Read Verdict)

Please notify the Court when you have reached a verdict, but do not tell anyone what your verdict is. You shall keep the verdict forms, these instructions and the exhibits until I give you further instructions.

## INSTRUCTION NO. 46

Once you begin your deliberations, if you have a question about the evidence in this case or about the instructions or verdict forms that you have been given, your Foreperson should write the question on a piece of paper, sign it and give it to the Marshal who will bring it to me.

I will then confer with the attorneys as to the appropriate way to answer your question. However there may be some questions that, under the law, I am not permitted to answer. If it is improper for me to answer the question, I will tell you that. Please do not speculate about what the answer to your question might be or why I am not able to answer a particular question.

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF COLORADO

Civil Action No. 99-Z-870

METABOLITE LABORATORIES, INC.
a Colorado corporation, and
COMPETITIVE TECHNOLOGIES, INC.,
a Delaware corporation,

Plaintiffs,

vs.

LABORATORY CORPORATION OF AMERICA
HOLDINGS d/b/a LabCorp, a Delaware corporation,

Defendant.

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**Special Verdict Form**
[Filed November 20, 2001]

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We, the jury, upon our oath, unanimously answer the questions in this Special Verdict Form as follows:

1. Do you find, by a preponderance of the evidence, that Defendant LabCorp is licensed under the '658 patent and that license has not been terminated in whole or in part?

_____ Yes __X__ No

**IF YOU ANSWERED "YES" TO QUESTION NO. 1, THEN ANSWER QUESTION NO. 2. IF YOU ANSWERED "NO" TO QUESTION NO. 1, THEN GO TO QUESTION NO. 5 AND 6.**

2. Do you find, by a preponderance of the evidence, that LabCorp breached the License Agreement with Plaintiff Metabolite by failing to pay Plaintiffs royalties on the Abbott homocysteine test?

_____ Yes _____ No

**IF YOU ANSWERED "YES" TO QUESTION NO. 2, ANSWER QUESTION NOS. 3 AND 4. IF YOU ANSWERED "NO" TO QUESTION NO. 2, DO NOT ANSWER QUESTION NOS. 3, 4, 5, 6, 7, 8 and 9, ANSWER QUESTION NOS. 10 AND 11.**

3. State the amount of royalties due, if any, to Plaintiff Metabolite, under the License Agreement, Section 2.02, which provides for a royalty of 21.5%. (Exhibit AC)

$_____

4. State the amount of royalties due, if any, to Plaintiff CTI under the License Agreement, Section 2.03, which provides for a royalty of 6%. (Exhibit AC)

$_____

**UPON COMPLETION OF QUESTION NOS. 3 AND 4, DO NOT ANSWER QUESTIONS NOS. 5, 6, 7, 8 AND 9 BUT ANSWER QUESTION NOS. 10 AND 11.**

5. Do you find, by a preponderance of the evidence, that LabCorp breached its license agreement by terminating it with respect to its performance of the Abbott test?

__X__ Yes _____ No

**IF YOU ANSWERED "YES" TO QUESTION NO. 5, THEN ANSWER THE REST OF THE QUESTIONS. IF YOU ANSWERED "NO" TO QUESTION NO 5, THEN GO TO QUESTION NO 10 AND 11.**

6. State the damages, if any, to plaintiff Metabolite for the breach of contract.

$3,652,724.61

7. Do you find, by a preponderance of the evidence, that Defendant LabCorp has contributed to or induced infringement of claim 13 of the '658 patent?

Induced infringement of Claim 13:

__X__ Yes _____ No

Contributed to infringement of Claim 13:

__X__ Yes _____ No

**IF YOU ANSWERED "YES" TO ANY PART OF QUESTION NO. 7, COMPLETE QUESTION NOS. 8 AND 9. IF YOU ANSWERED "NO" TO BOTH PARTS OF QUESTION NO. 7, THEN DO NOT ANSWER QUESTION NOS. 8 AND 9 AND ANSWER QUESTION NOS. 10 AND 11.**

8. State the amount of damages, if any, to Plaintiff CTI caused by Defendant LabCorp's infringement by inducement or by contributory infringement.

$1,019,365.01

9. Do you find, by clear and convincing evidence, that LabCorp has willfully infringed claim 13 of the '658 patent?

__X__ Yes _____ No

**YOU MUST ANSWER QUESTION NOS. 10 and 11.**

10. Do you find, by clear and convincing evidence, that claims 13 or 18 of the '658 patent are invalid?

_____ Yes __X__ No

**IF YOU ANSWERED "YES" STATE WHICH CLAIMS ARE INVALID:**

| | |
|---|---|
| Invalid for Nonenablement: | Claim 13 _____ |
| | Claim 18_____ |
| Invalid for Insufficient Written Description: | Claim 13_____ |
| | Claim 18_____ |
| Invalid for Indefiniteness: | Claim 13_____ |
| | Claim 18_____ |
| Invalid for Obviousness: | Claim 13_____ |
| | Claim 18_____ |
| Invalid for Anticipation: | Claim 13_____ |

11. Do you find, by clear and convincing evidence, that:

(a) CTI or one of the inventors of the '658 patent withheld information or misrepresented information that was material to the patent examiner's examination of the claims of the patent?

_____ Yes __X__ No

(b) CTI or one of the inventors of the '658 patent withheld material information or made material misrepresentations with the intent to deceive the patent examiner in allowing the '658 patent to issue?

_____ Yes __X__ No

After answering the appropriate questions, the foreperson should date and sign the verdict form.

Dated: November 20, 2001

/s/ John MacKay
FOREPERSON

[Filed Dec. 3, 2001]

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF COLORADO

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Civil Action No. 99-Z-870

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METABOLITE LABORATORIES, INC.,
a Colorado corporation, and
COMPETITIVE TECHNOLOGIES, INC.,
a Delaware corporation,

Plaintiffs,

v.

LABORATORY CORPORATION OF AMERICA
HOLDINGS d/b/a LABCORP,
a Delaware corporation,

Defendant.

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**JUDGMENT**

This matter came on for trial before the Court and a jury of eight beginning on November 5, 2001, the Honorable Zita L. Weinshienk, United States District Judge, presiding.

At the conclusion of all of the evidence, the jury returned its Special Verdict Form on November 20, 2001, which is incorporated herein by reference as if fully set forth. Accordingly, it is

ORDERED that a judgment is entered in favor of the plaintiffs Metabolite Laboratories, Inc. and Competitive Technologies and against defendant Laboratory Corporation of America Holdings. It is

FURTHER ORDERED that the damages to be awarded to plaintiff Metabolite for defendant LabCorp's breach of contract are $3,652,724.61. It is

FURTHER ORDERED that the damages to be awarded to plaintiff Competitive Technologies, Inc., due to defendant LabCorp's infringement by inducement or by contributory infringement are $1,019,365.01. It is

FURTHER ORDERED that post-judgment interest shall accrue at the legal rate of 2.23% per annum from the date of entry of judgment.

DATED, at Denver, Colorado, this 3 day of December, 2001.

BY THE COURT:

/s/ Zita L. Weinshienk
ZITA L. WEINSHIENK, Senior Judge
United States District Court

[Filed Nov. 20, 2002]

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF COLORADO

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Civil Action No. 99-Z-870

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METABOLITE LABORATORIES, INC.,
a Colorado corporation, and
COMPETITIVE TECHNOLOGIES, INC.,
a Delaware corporation,

Plaintiffs,

v.

LABORATORY CORPORATION OF AMERICA
HOLDINGS d/b/a LABCORP,
a Delaware corporation,

Defendant.

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**AMENDED JUDGMENT**

This matter came on for trial before the Court and a jury of eight beginning on November 5, 2001, the Honorable Zita L. Weinshienk, United States District Judge, presiding.

At the conclusion of all of the evidence, the jury returned its Special Verdict Form on November 20, 2001, which is incorporated herein by reference as if fully set forth. Accordingly, it is

ORDERED that a judgment is entered in favor of the Plaintiffs Metabolite Laboratories, Inc. and Competitive Technologies and against Defendant Laboratory Corporation of America Holdings. It is

FURTHER ORDERED that damages are awarded to Plaintiff Metabolite for Defendant LabCorp's breach of contract in the amount of $3,652,724.61. It is

FURTHER ORDERED that damages are awarded to Plaintiff Competitive Technologies, Inc., due to Defendant LabCorp's infringement by inducement or by contributory infringement in the amount of $1,019,365.01. It is

**FURTHER ORDERED that enhanced damages are awarded to Plaintiff Competitive Technologies, Inc. in the amount of $1,019,365.01. It is**

**FURTHER ORDERED that Plaintiff Metabolite Laboratories, Inc. is awarded $473,946.97 in prejudgment interest. It is**

**FURTHER ORDERED that Plaintiff Competitive Technologies, Inc. is awarded $132,264.27 in prejudgment interest.** It is

FURTHER ORDERED that post-judgment interest shall accrue at the legal rate of 2.23% per annum from the date of entry of judgment.

DATED at Denver, Colorado, this 19 day of November, 2002.

BY THE COURT:

/s/ Zita L. Weinshienk
ZITA L. WEINSHIENK, Senior Judge
United States District Court

# Supreme Court of the United States

No. 04-607

LABORATORY CORPORATION OF AMERICA HOLDINGS, dba LabCorp,

Petitioner,

v.

METABOLITE LABORATORIES, INC., et al.,

Respondents

Order of October 31, 2005

Petition for writ of certiorari to the United States Court of Appeals for the Federal Circuit granted limited to Question 3 presented by the petition.

# Supreme Court of the United States

No. 04-607

LABORATORY CORPORATION OF AMERICA HOLDINGS, dba LabCorp,

Petitioner,

v.

METABOLITE LABORATORIES, INC., et al.,

Respondents

Order of November 2, 2005

Having been advised by THE CHIEF JUSTICE that he now realizes that he should have recused himself from participation in this case, and does now recuse himself, the Court vacates its order of Monday, October 31, 2005. The Court has reconsidered the petition for certiorari, which is granted but limited to question three as presented in the petition. THE CHIEF JUSTICE has not participated in the vote to withdraw the order of October 31, 2005, or in the instant reconsideration of the petition for certiorari.

of the patent." *Manual of Patent Examining Procedure* § 2173.02. Petitioner's indefiniteness challenge is that "[t]he claim as construed says *nothing* at all about what it means to actively 'correlate' a test result." Pet. Br. 39. This argument cannot be reconciled with the record or petitioner's own litigating position in the lower courts.

In the district court, *petitioner* proposed that "correlating" means to "establish a mutual or reciprocal relationship." Pet. App. 7a-8a. The district court adopted that proposed construction and then petitioner stipulated to it in a jury instruction:

> A method for determining the existence of a shortage of cobalamin or folate necessary to health in warm-blooded animals comprising the steps of 1) assaying a body fluid for a level of total homocysteine raised above the normal range and 2) establish[ing] a mutual or reciprocal relationship between a level of total homocysteine raised above the normal range in said body fluid with a shortage of cobalamin or folate necessary to health[,] the latter step describ[ing] a discrete step in a sequential process.

J.A. 376-377 (internal quotations omitted). The Federal Circuit agreed that establishing a mutual or reciprocal relationship was a sufficiently definite description. Pet. App. 16a; *see also* U.S. Br. 14 ("[C]laim 13 satisfies the definiteness requirement because it marks the boundaries of the patent claim with precision. . . . Although [the claim] language is undeniably sweeping, it is not unclear.").

Petitioner presented no evidence at trial to support its current assertion that the correlating step is unclear. To the contrary, its own medical expert, its own Discipline Director, and its own Laboratory Director all testified that the step was quite clear to them. J.A. 154, 150-151, 162-164, 112-113. In its jury instruction contentions, petitioner failed to assert that

the correlating step was unclear. J.A. 365-366, 385-388. In its opening statement and closing argument, petitioner never asserted that the correlating step was unclear. Trial Tr. 131-141, 1772-1798.

Petitioner itself has published extensive materials explaining the mutual and reciprocal relationship between total homocysteine and cobalamin and folate deficiencies. *See* J.A. 189-195, 255-256, 257-258, 259-261. Petitioner's literature acknowledges that the Inventors discovered "the clinical correlations and analytical methodology," and in particular that they had patented "methods for detecting and distinguishing cobalamin and folic acid deficiency." J.A. 196. Until it stopped paying royalties, petitioner never questioned the validity of the '658 patent on any grounds.

Now, as if the burden were on respondents to disprove petitioner's bald contention that *its own* construction of "correlating" is unclear, petitioner protests that "nothing . . . tells a practitioner how to actively 'establish' a 'relationship' between a particular test result and a vitamin deficiency." Pet. Br. 39. This assertion cannot be reconciled with the specification itself, which teaches that "[h]omocysteine levels above [the normal] ranges are indicative of cobalamin and/or folate deficiency; the higher the level, the stronger the indication." S.A. 14; *see also* S.A. 11. The patent goes on to quantify the total homocysteine ranges in the sample populations and to describe in detail the procedures by which the Inventors established the mutual and reciprocal relationship between total homocysteine and vitamin deficiency.[7] As the Federal Cir-

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[7] Example I, Example VI, and Example VII all describe the Inventors' extensive patient studies, in which they established that total homocysteine was "diagnostically useful" in evaluating vitamin deficiencies and "more sensitive" than previous tests. S.A. 14-15, 27-29. These examples parallel the papers authored by the Inventors and published—after peer review—in prestigious medical journals. C.A. App. 8885-8895, 8644-8652, 8862-8871.

cuit explained, "the record shows repeatedly that the correlating step is well within the knowledge of one of skill in this art." Pet. App. 18a. Petitioner has not demonstrated any error in that factual determination.

### B. The Specification Contains A Sufficient Written Description And Enables One Skilled In The Art To Practice The Invention

The jury and lower courts correctly concluded that the specification also contains a sufficient written description of the invention and enables a person skilled in the art to make and use the invention. J.A. 396-397; Pet. App. 34a-35a, 17a-18a.

1. The purpose of the written description requirement is to "convey to a person of skill in the art that the patentee had possession of the claimed invention at the time of the application, i.e., that the patentee invented what is claimed." *LizardTech, Inc.* v. *Earth Res. Mapping, Inc.*, 424 F.3d 1336, 1345 (Fed Cir. 2005); *see Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶1, "Written Description" Requirements*, 66 Fed. Reg. 1099, 1104 (Jan. 5, 2001). As with its definiteness challenge, petitioner's sole challenge to the written description of the invention is that "nothing in the specification says exactly what [the correlating step] includes or how to do it." Pet. Br. 41. To the contrary, the specification explains that "[i]t has been discovered that elevated levels of homocysteine in body tissue correlate with decreased levels of cobalamin and/or folic acid in said body tissue." S.A. 12. The patent recites several prior-art methods of assaying for total homocysteine, but notes that "[t]he procedure has never been used to monitor homocysteine to detect or measure cobalamin or folic acid deficiency." *Ibid.*

The patent clearly describes how the Inventors practiced the correlating step en route to inventing the diagnostic

method of claim 13. *See* note 7 and accompanying text, *supra*; U.S. Br. 9 ("the patent specification easily satisfies the . . . written description requirement[] by . . . demonstrating that the applicants had in fact performed [the claimed method]"). Moreover, the Federal Circuit explained that "the PTO read the specification to include [the correlating] feature," and "the record reflects that [petitioner's] own expert and employees understood the meaning of 'correlating.'" Pet. App. 17a. Petitioner has not challenged either of these factual determinations as clearly erroneous—indeed, petitioner does not take issue with any aspect of the lower courts' rejection of its written description defense. Because the written description issue is "primarily factual" (*Union Oil Co.* v. *Atlantic Richfield Co.*, 208 F.3d 989, 1005 (Fed. Cir. 2000); *see* U.S. Br. 11), and "[t]he record is replete with evidentiary support" that one skilled in the art would "underst[and] from the specification that the '658 patent inventors possessed the 'correlating' step" (Pet. App. 17a), petitioner's written description argument fails.

2. The specification also easily meets the enablement requirement, which "is satisfied when one skilled in the art, after reading the specification, could practice the claimed invention without undue experimentation." *AK Steel Corp.* v. *Sollac & Ugine*, 344 F.3d 1234, 1244 (Fed. Cir. 2003).

Petitioner's sole enablement challenge is that "nothing in the specification informs a skilled artisan what [the correlating step] is." Pet. Br. 41. This is wrong for the reasons just discussed: Since the specification describes in detail how the Inventors practiced the correlating step, it would enable one skilled in the art to replicate (or build upon) their research.

In fact, the specification describes in great detail the manner in which to conduct assays and correlations. For example, the specification describes "several different known assays suitable for use in determining levels of homocysteine in urine or blood," S.A. 12, as well as a new assay method

claimed in the '658 patent, S.A. 12-14. As the Federal Circuit explained, "the record shows repeatedly that the correlating step is well within the knowledge of one of skill in this art." Pet. App. 18a; *see* U.S. Br. 9 ("Especially from the perspective of such a person, the patent specification easily satisfies the enablement . . . requirement[] by explaining precisely how to perform the claimed method"). Petitioner has not even challenged this factual conclusion.

## II. Petitioner's Contention That Claim 13 Does Not Recite Patentable Subject Matter Is Not Properly Presented, And In Any Event Is Meritless

Section 101 of the Patent Act provides that "*[w]hoever invents or discovers any new and useful process*, machine, manufacture, or composition of matter, *may obtain a patent therefor*, subject to the conditions and requirements of this title." 35 U.S.C. § 101 (emphases added). As this Court has recognized (*Diamond* v. *Chakrabarty*, 447 U.S. 303, 315 (1980)), Section 101 is the congressional implementation of the constitutional authority to "promote the Progress of Science and useful Arts, by securing for limited Times to . . . Inventors the exclusive Right to their . . . Discoveries." U.S. Const. art. I, § 8, cl. 8. Contrary to petitioner's unsupported assertion that "[t]here is a longstanding and key distinction between a potentially useful scientific *discovery* and a patentable *invention*" (Pet. Br. 31), the Patent Act unequivocally states that "[t]he term 'invention' means invention or discovery." 35 U.S.C. § 100(a); *see Corning* v. *Burden*, 56 U.S. (15 How.) 252, 268 (1854) ("A new process is usually the result of discovery; a machine, of invention").

The Inventors "invent[ed] or discover[ed]" a "new and useful process"—a novel method for detecting cobalamin and folate deficiencies that often went undiagnosed using traditional methods. *See* Pet. App. 2a-3a; S.A. 12, 29. Claim 13 of the '658 patent, which recites that method, is "presumed valid." 35 U.S.C. § 282; *see Cardinal Chem. Co.* v.

*Morton Int'l, Inc.*, 508 U.S. 83, 93 n.15 (1993). Moreover, "[t]he burden of establishing invalidity . . . shall rest on the party asserting such invalidity." 35 U.S.C. § 282; *see also Budde* v. *Harley-Davidson, Inc.*, 250 F.3d 1369, 1381 (Fed. Cir. 2001) (invalidity must be established by "clear and convincing evidence"); Pet. App. 15a. Petitioner has not met that heavy burden. Indeed, in the courts below it did not even try.

### A. Subject Matter Patentability Is Not Properly Before The Court

The thrust of petitioner's submission in this Court is that "Claim 13 runs afoul" of "the 'established rule'" that "a scientific fact 'cannot be the subject of a patent.'" Pet. Br. 21 (quoting *Parker* v. *Flook*, 437 U.S. 584, 589 (1978)). Accordingly, petitioner's challenge "turns entirely on the proper construction of § 101 of the Patent Act, which describes the subject matter that is eligible for patent protection." *Flook*, 437 at 588; *see also Diehr*, 450 U.S. at 181-82, 185, 188-89, 191-92.[8] Yet, with the exception of a single cryptic footnote in its merits brief filed in this Court (Pet. Br. 19-20 n.10), petitioner has *never* so much as cited, much less invoked or discussed, Section 101 in the long history of this litigation.

Almost in passing, petitioner maintains that subject matter patentability is so important that it can be raised at any time in the litigation, even if it was not pleaded in the answer. Pet. Br. 20 & n.11. For this proposition, petitioner cites two 19th Century cases that state that non-patentability need not be pleaded. *Hill* v. *Wooster*, 132 U.S. 693, 698 (1890); *Slawson* v. *Grand Street R.R. Co.*, 107 U.S. 649, 652 (1883); *see*

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[8] Petitioner's *amici* uniformly understand its challenge to be based on Section 101. *See, e.g.*, AARP Br. 26 ("There is no patentable *invention* in Claim 13 under § 101"); ACLA Br. 16 ("[E]ven when claim 13 is viewed as a whole, it still fails to describe a 'process' within the meaning of 35 U.S.C. § 101").

*also, e.g., Hendy* v. *Golden State & Miners' Iron Works*, 127 U.S. 370, 375 (1888). Unfortunately for petitioner, the proposition reflected in those cases has been thrice abrogated in the last century: Not only did Congress expressly reject it in the Patent Act of 1952 as a matter of substantive patent law, but both this Court's 1937 promulgation of the Federal Rules of Civil Procedure and related rules for appellate courts also foreclose it as a matter of federal procedural law.

### 1. Petitioner Failed To Plead Or Prove A Non-Patentability Defense As Required By The Patent Act

During the late 19th Century when petitioner's authorities were decided, the Patent Act set forth five specific defenses that defendants in infringement actions were required to plead and prove at trial. Patent Act of 1870, ch. 230, § 61, 16 Stat. 198, 208 (July 8, 1870) (R.S. 4920). If one of the five enumerated defenses was not included in an answer, courts would not allow a party to raise the defense at a later time. *E.g., Guidet* v. *Barber*, 11 F. Cas. 103, 104 (C.C.D.N.J. 1873) (No. 5,857) (holding that a defense based on lack of novelty falls within enumerated defenses and cannot be raised if not "specified" in the answer). Significantly, the five enumerated defenses set forth in the 1870 version of the Patent Act did *not* call on defendants to plead lack of patentable subject matter as a defense. Accordingly, even courts that refused to consider one of the five enumerated defenses would excuse a defendant's failure to raise subject matter patentability in his answer. *Ibid.*

In 1952, Congress revised the Patent Act and broadened the range of defenses to be pleaded in infringement cases. New Section 282 replaced what had been Section 61 of the 1870 version of the Patent Act (R.S. 4920) with a broader, more inclusive list of defenses required to be pleaded. *See* Reviser's Notes, 1952 U.S.C.C.A.N. 2394, 2422 ("The five defenses named in R.S. 4920 are omitted and replaced by a

broader paragraph specifying defenses in general terms"). For the first time, Congress included among the defenses that "shall be pleaded" the defense of non-patentable subject matter under Section 101. 66 Stat. 792, 812 (codified at 35 U.S.C. § 282). The principle of *Slawson* and similar cases—that federal courts could consider subject matter patentability even when the defense was not raised in an answer—did not survive this substantive revision to the Patent Act.

Thus, the Patent Act now provides, in no uncertain terms, that "[t]he following shall be defenses in any action involving the validity or infringement of a patent *and shall be pleaded*: . . . Invalidity of the patent or any claim in suit on any ground specified in part II of this title as a condition for patentability." 35 U.S.C. § 282 (emphasis added). "Part II" of title 35 includes the Section 101 requirements of subject matter patentability. *Standard Oil Co.* v. *Am. Cyanamid Co.*, 774 F.2d 448, 453 (Fed. Cir. 1985).

Petitioner failed to plead a lack of subject matter patentability under Section 101 in its answer. *See* J.A. 65-70.[9] Petitioner's answer includes assertions that claim 13 is invalid for failure to satisfy Sections 102 (novelty), 103 (nonobviousness), and 112 (definiteness), but the answer does not contain any assertion that claim 13 recites unpatentable subject matter or otherwise is invalid under Section 101. J.A. 66, 64-82. Moreover, petitioner introduced no evidence on this issue at trial; the jury was not charged on subject matter patentability, and it did not return a verdict on any Section 101 defense. *See* J.A. 362-393 (complete set of jury instructions); 394-397 (special verdict form).

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[9] Although petitioner maintains without elaboration that "the issue was raised below" (Pet. Br. 17 n.9), petitioner never even mentioned Section 101 or non-patentable subject matter in the lower courts. U.S. Cert. Br. 15 ("Indeed, petitioner did not mount any challenge, under any theory, to the patentability of the claimed subject matter under Section 101").

As this Court has recognized in numerous contexts, courts must strictly enforce statutory prerequisites to litigation. *See, e.g.*, *Hallstrom* v. *Tillamook County*, 493 U.S. 20, 33 (1989); *McNeil* v. *United States*, 508 U.S. 106, 113 (1993). This is especially important where, as is the case with patents, such requirements manifestly are designed to ensure that complex questions of law, fact, science and policy are fully vetted *at trial*. As Justice Breyer has observed, it is essential that courts "mak[e] difficult science-related choices only when there has been extensive, informed development of the relevant legal and policy issues *prior* to decision." Breyer, *Genetic Advances and Legal Institutions*, 28 J. L. Med. & Ethics 23, 23 (2000). Here, because petitioner failed to include a Section 101 defense in its answer, petitioner cannot now assert that the '658 patent fails the requirements of subject matter patentability. *See, e.g.*, *Elec. Storage Battery Co.* v. *Shimadzu*, 307 U.S. 5, 16-17 (1939) (refusing to consider a validity defense that an infringer failed to plead or prove in the lower courts); *Johnson & Johnson* v. *C.B. Stenvall, Inc.*, 193 F. Supp. 128, 132 (S.D.N.Y. 1961) ("[E]ven if the defense of abandonment had merit, it was neither pleaded in the answer nor noticed in writing at least 30 days before the trial as required by 35 U.S.C. § 282, and therefore is not entitled to consideration").

### 2. Petitioner's Failure To Plead A Non-Patentability Defense Runs Afoul Of General Pleading Rules

Petitioner's belated attempt to raise the Section 101 patentability defense also violates the Federal Rules of Civil Procedure, which expressly require defendants to plead "any . . . matter constituting an avoidance or affirmative defense." Fed. R. Civ. P. 8(c). As Professors Wright and Miller have observed, this rule has led the lower courts "virtually universal[ly]" to conclude that "a failure to plead an affirmative defense . . . results in the waiver of that defense and its exclu-

sion from the case." 5 *Federal Practice & Procedure* § 1278 (3d ed. 2004). Barely two years ago, in *Kontrick* v. *Ryan*, 540 U.S. 443 (2004), this Court agreed.

This Court held in *Kontrick* that a debtor forfeited his right to rely on a defense by failing to raise the issue in his answer or, indeed, at any time before the trial court reached the merits of the case. *See* 540 U.S. at 458-60. The Court made clear that "under the Bankruptcy Rules *as under the Civil Rules*, a defense is lost if it is not included in the answer or amended answer" or, "at the latest, 'at the trial on the merits.'" *Id.* at 459-60 (quoting Fed. R. Civ. P. 12(h)(2)) (emphasis added); see also *Hormel*, 312 U.S. at 556 ("our procedural scheme contemplates that parties shall come to issue in the trial forum vested with authority to determine questions of fact"). Here, as in *Kontrick*, petitioner is barred from raising the Section 101 patentability affirmative defense after it lost its case on the merits in the district court.

Petitioner further cemented the forfeiture of the Section 101 defense by failing to raise the issue at all until the case reached the merits stage at this Court. *See Helvering* v. *Tex-Penn Oil Co.*, 300 U.S. 481, 497-98 (1937) (petitioner "sought no ruling upon the question from the board or the lower court and is therefore not entitled to have it decided here"). Petitioner, represented by sophisticated counsel throughout this litigation, had multiple opportunities to attempt to inject the Section 101 invalidity argument into the case, and with each pleading, brief, or other submission petitioner knowingly bypassed that argument. At trial, petitioner put forth evidence and argument to support the invalidity arguments it had pleaded, but never mentioned Section 101 or subject matter patentability. No witness testified on the issue. No jury instruction was given regarding patentable subject matter or Section 101. Petitioner's pre-submission and post-trial motions for judgment as a matter of law similarly failed to raise a Section 101 defense. Dkt. 249, 250. As this Court recently reaffirmed in *Unitherm Food Systems, Inc.* v.

*Swift-Eckrich, Inc.*, No. 04-597, slip op. 5-9, this last failure *by itself* was sufficient to deprive the Federal Circuit and this Court of any "power" to grant petitioner *any* relief (*i.e.*, reversal or a new trial), even if the Section 101 argument had been properly raised on appeal thereafter. *See also Cone* v. *W. Va. Pulp & Paper Co.*, 330 U.S. 212, 217-18 (1947); *Hopp v. City of Pittsburgh*, 194 F.3d 434, 440 (3d Cir. 1999) (Alito, J.).

Petitioner, of course, did *not* thereafter appeal any issue related to Section 101 or subject matter patentability to the Federal Circuit. Instead, petitioner reiterated its invalidity arguments under Sections 102, 103, and 112; it made no mention of Section 101 in its opening brief, its reply brief, or its petition for rehearing. In the court below, as in every other court of appeals, petitioner's failure to brief the claim it now seeks to present to this Court is, in and of itself, an independent basis for deeming that claim forfeited. *E.g.*, *Pandrol USA, LP* v. *Airboss Ry. Prods., Inc.*, 320 F.3d 1354, 1366 n.3 (Fed. Cir. 2003); *Becton Dickinson & Co.* v. *C.R. Bard, Inc.*, 922 F.2d 792, 800 (Fed. Cir. 1990).[10]

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[10] Petitioner clings to a passage in its opening Federal Circuit brief to suggest that it raised the issue below. Pet. Br. 14 (quoting Pet. C.A. Br. 41). The desperate implausibility of this contention can scarcely be overstated. Although petitioner cited *Diehr* for the proposition that natural phenomena are excluded from patent protection, it did *not* challenge the validity of the patent under Section 101. *See* Pet. C.A. Br. 38. Indeed, after *respondents* pointed out in their answering Federal Circuit brief that petitioner had waived any conceivable Section 101 argument (Resp. C.A. Br. 71), petitioner said nothing further on the point. Thus, not only did petitioner fail to make an affirmative Section 101 argument, it also tellingly declined to engage on the question when respondents noted the waiver of any such issue.

### 3. This Court's Own Rules Preclude Consideration Of A Defense Of Non-Patentability

Petitioner nonetheless contends that this Court should review the Section 101 issue because that issue is "fairly included in Question 3." Pet. Br. 17 n.9. Even if that were true, it would not excuse the fact that the patentability issue was never raised in, or decided by, the courts below. *See, e.g., Bankers Life & Cas. Co.* v. *Crenshaw*, 486 U.S. 71, 76-77 (1988) (treating issue "as if contained in a petition for a writ of certiorari," but "declin[ing] to decide the merits of the issue"). This Court is not a tribunal of "first view." *Adarand Constructors, Inc.* v. *Mineta*, 534 U.S. 103, 110 (2001) (quoting *Matsushita Elec. Indus. Co.* v. *Epstein*, 516 U.S. 367, 399 (1996) (opinion of Ginsburg, J.)).

In any event, the Section 101 issue is not "fairly included" in Question 3, which seeks review of the lower courts' determinations that claim 13 meets the requirements of Section 112. As this Court made clear in *Diehr*, Section 101's subject matter eligibility requirements are wholly *separate* from those set forth elsewhere, such as the novelty requirement of Section 102, the nonobviousness requirement of Section 103, or, by logical extension, the descriptive requirements of Section 112. 450 U.S. at 189-91; *see* U.S. Br. 14 (The "limitation under Section 101" on monopolizing natural phenomena "is entirely separate and distinct from the requirements of Section 112"). In light of *Diehr*, any Section 101 issue is sufficiently tangential to the Section 112 question presented as to make subject matter patentability *not* "fairly included therein." *Yee* v. *City of Escondido*, 503 U.S. 519, 537 (1992). Section 112's requirements and Section 101's requirements may "exist side by side," but "neither encompass[es] the other." *Ibid.*

Nor do any passing arguments in the *text* of the petition concerning the potential *consequences* of upholding the Fed-

eral Circuit's Section 112 rulings somehow serve to bring the Section 101 issue within Question 3. *See* U.S. Br. 15-16. To begin with, petitioner mentioned *Diehr* only to argue that it should not be held liable for *induced* infringement because the patent allegedly incorporates a "scientific fact"—a question on which this Court denied certiorari. Pet. 18-19. Moreover, even if a separate Section 101 invalidity argument *had* been included in the text of the petition—which it was not—that would not be sufficient to bring the issue before the Court under Rule 14.1(a), which "requires that a subsidiary question be fairly included in the *question presented* for [the Court's] review." *Izumi Seimitsu Kogyo Kabushiki Kaisha* v. *U.S. Philips Corp.*, 510 U.S. 27, 31 n.5 (1993).

In fact, it is abundantly clear that a Section 101 argument did not even occur to petitioner until the Court *sua sponte* sought the Solicitor General's views on such an argument, well after the petition and the opposition were filed. *See* Resp. Br. in No. 04-1579, at 2-3 n.*. For that reason, petitioner is wrong to contend that respondents should have opposed certiorari in this case on the ground that no Section 101 issue is properly before the Court. As the Solicitor General recognizes, nothing in this Court's rules required that respondents object, preemptively, to an issue that is not encompassed within any question presented. U.S. Br. 17; *see City of Springfield* v. *Kibbe*, 480 U.S. 257, 260 (1987). Because petitioner's Section 101 argument is not fairly encompassed within the question presented, and was neither pressed nor passed upon below, this Court should decline to consider it at this late date. And because that issue is the principal focus of the briefs filed by petitioner and its *amici*, this Court may wish to consider dismissing the writ of certiorari. *See, e.g.*, *Adarand*, 534 U.S. at 111; *City of Springfield*, 480 U.S. at 259-60.[11]

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[11] Dismissal would be particularly appropriate because the '658 patent will expire in July 2007. The Section 101 issue that

### B. The '658 Patent Claims Patentable Subject Matter

Assuming *arguendo* that the issue of subject matter patentability is properly before the Court, claim 13 of the '658 patent easily satisfies the requirements of Section 101. The Inventors discovered what could be considered, standing alone, a natural phenomenon, and then put that discovery to practical use by inventing a method for diagnosing cobalamin or folate deficiencies. In a line of cases culminating with *Diehr*, this Court has held that such "an *application* of a law of nature or mathematical formula to a known structure or process may well be deserving of patent protection." 450 U.S. at 187 (emphasis in original); *see also, e.g., Chakrabarty*, 447 U.S. at 309-10; *Mackay Radio & Tel. Co.* v. *Radio Corp. of Am.*, 306 U.S. 86, 94 (1939); *Le Roy* v. *Tatham*, 55 U.S. (14 How.) 156 (1853).

Since *Diehr* was decided in 1981, the Federal Circuit has been the final arbiter on questions of subject matter patentability. During that time, the Federal Circuit has applied *Diehr* in many cases to determine whether claimed processes employing natural phenomena fall within the statutory subject matter set forth in Section 101. *See, e.g., AT&T Corp.* v. *Excel Communications, Inc.*, 172 F.3d 1352 (Fed. Cir. 1999); *State Street Bank & Trust Co.* v. *Signature Fin. Group, Inc.*,

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petitioner failed to raise during five years of litigation would have little practical significance just a year after this Term ends. At that time, the injunction entered by the district court (which has been stayed pending resolution of appellate proceedings) will also terminate. Although petitioner and some *amici* express concern with the scope of that injunction (*see* U.S. Br. 23-24, 29; Pet. Br. 36-37; AARP Br. 16), that concern—like petitioner's contention (Br. 48 n.28) that "the injunction should be vacated in light of whatever standard the Court announces in *eBay, Inc.* v. *MercExchange, L.L.C.*, No. 05-130"—is misplaced: Petitioner did not challenge the injunction in its petition, and the Court did not grant review of the scope of the injunction.

149 F.3d 1368 (Fed. Cir. 1998); *In re Alappat*, 33 F.3d 1526 (Fed. Cir. 1994) (en banc). Today, it is clear that Section 101's subject matter requirements are met if a natural phenomenon incorporated in the claimed invention "has been reduced to some practical application rendering it 'useful.'" *Excel*, 172 F.3d at 1356-57; *accord Alappat*, 33 F.3d at 1543-44; *State Street*, 149 F.3d 1373.

The PTO has followed this Court's and the Federal Circuit's precedent regarding Section 101, and its *Interim Guidelines for Examination of Patent Applications for Patent Subject Matter Eligibility*, 1300 Off. Gaz. 142 (Nov. 22, 2005)—which "are believed to be fully consistent with binding precedent of the Supreme Court, the Federal Circuit and the Federal Circuit's predecessor courts" (App., *infra*, 5a)—are highly instructive.[12] Those *Guidelines* set forth the methodology that patent examiners are to use in determining whether patent applications claim statutory subject matter. *See* App., *infra*, 20a (flowchart summarizing steps in the analysis). As they are entitled at the very least to deference under *Skidmore* v. *Swift & Co.*, 323 U.S. 134, 140 (1944), the *Guidelines* (which petitioner fails even to mention) also provide a useful framework for judicial analysis of subject matter patentability.

### 1. The Claimed Invention Falls Within A Category Enumerated In 35 U.S.C. § 101

The expansive language of Section 101 allows a patent on "anything under the sun that is made by man." *Chakrabarty*, 447 U.S. at 308-09 (quoting S. Rep. No. 1979, at 5

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[12] Because this volume of the *Official Gazette* is not yet available in print, we have reproduced pertinent portions of the PTO's *Subject Matter Eligibility Guidelines* in the appendix to this brief. *See also* 70 Fed. Reg. 75,451, 75,452 (Dec. 20, 2005) (requesting public comment on the *Guidelines*).

(1952), H.R. Rep. No. 1923, at 6 (1952)). In addition to machines, manufactures, and compositions of matter, Section 101 makes "processes" patentable. "A process is a mode of treatment of certain materials to produce a given result." *Cochrane* v. *Deener*, 94 U.S. 780, 788 (1877); *accord Diehr*, 450 U.S. at 183. Claim 13 fits this description precisely: It claims a process for treating certain materials (*i.e.*, assaying body fluids and correlating the assay results with vitamin status) to achieve a desired result (*i.e.*, detecting cobalamin or folate deficiencies).

Contrary to petitioner's assertion (Br. 23), it is of no moment that the patented process can be practiced using an assay procedure that may have been known previously.[13]

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[13] The government briefly questions whether claim 13 might be invalid under 35 U.S.C. § 102 (anticipation) "because the claim effectively prevents doctors from using previously known assay methods to measure total homocysteine for *any* purpose, even if the purpose was not to diagnose cobalamin or folate deficiency." U.S. Br. 28. There are indeed other purposes for assaying for total homocysteine, such as detecting certain inherited enzyme defects (which are relatively rare). S.A. 14 ("When homocysteine levels are elevated in individuals *without inherited defects*, at least one of folate or cobalamin is deficient") (emphasis added); *see also* S.A. 11; J.A. 137; C.A. App. 9648, 9697, 5552. The injunction in this case does not prevent "doctors" from making use of such assays. Rather, it precludes *petitioner*—which is not a doctor—from assaying for total homocysteine because *petitioner* failed to offer any evidence that any of *its* assays were for any of these other purposes. In any event, as the government concedes, the anticipation issue is "not before this Court." U.S. Br. 28. At trial, petitioner raised an anticipation defense, which was rejected by the jury. J.A. 396-397. Petitioner reiterated its anticipation defense on appeal, where it was rejected by the Federal Circuit. Pet. App. 18a-19a. Petitioner did not seek certiorari on the anticipation issue, so it has been abandoned. *Posters 'N' Things, Ltd.* v. *United States*, 511 U.S. 513, 527 (1994). There is no reason, or basis, to consider or decide an issue raised only in passing by the United States as *amicus curiae*. *See, e.g.*, *United Parcel Service, Inc.* v. *Mitchell*,

"The process requires that certain things should be done with certain substances, and in a certain order; but the tools to be used in doing this may be of secondary consequence." *Cochrane*, 94 U.S. at 788. Claim 13 teaches that doing "certain things" (assaying and correlating) with "certain substances" (body fluids) in a "certain order" (sequentially) will achieve a desired result—the diagnosis of vitamin deficiency. That is the essence of a patentable method. *Corning*, 56 U.S. (15 How.) at 268 ("It is when the term process is used to represent the means or method of producing a result that it is patentable").

It is therefore clear that claim 13 recites a process, and thus that it is drawn to statutory subject matter. The remaining question is whether the invention falls within one of the exceptions that this Court has recognized to Section 101's intentionally broad scope.

### 2. The Claimed Invention Does Not Fall Within A Judicial Exception To Section 101

"This Court has . . . recognized limits to § 101 and every discovery is not embraced within the statutory terms. Excluded from such patent protection are laws of nature, natural phenomena, and abstract ideas." *Diehr*, 450 U.S. at 185. These categories constitute "judicially created exception[s] to § 101." *Alappat*, 33 F.3d at 1542.

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451 U.S. 56, 60 n.2 (1981). In any event, the government's conjecture is mistaken. The PTO in fact rejected a previous iteration of claim 13 "as being anticipated" by prior art assays "[i]n the absence of a correlation step." J.A. 285. In response, the Inventors amended claim 13 "to recite a second step of correlating an elevated level of total homocysteine with a deficiency of cobalamin or folate." J.A. 290. The PTO then *withdrew* its Section 102 objection and allowed the patent as amended to issue. *See* Pet. App. 9a (summarizing prosecution history).

The correlation between total homocysteine and deficiencies in cobalamin and folate that the Inventors discovered could be considered, standing alone, a "natural phenomenon" in the literal sense: It is an observable aspect of biochemistry in at least some human populations. Of course, in the physical world "[e]verything that happens may be deemed 'the work of nature.'" *Funk Bros. Seed Co.* v. *Kalo Inoculant Co.*, 333 U.S. 127, 135 (1948) (Frankfurter, J., concurring). The Court has held that "[h]e who discovers a hitherto unknown phenomenon of nature has no claim to a monopoly of it which the law recognizes." *Id.* at 130 (majority opinion). But respondents do not claim a monopoly to the correlation discovered by the Inventors. Rather, claim 13 recites a *method* for using the correlation as a sequential step toward achieving the desirable end of diagnosing vitamin deficiencies. It is clearly patentable under existing law.

### a. The Claimed Invention Covers A Practical Application Of A Natural Phenomenon

In *Flook*, the Court took a restrictive view of patentable subject matter and concluded that a claimed invention must include a novel or inventive aspect separate and apart from the discovery of a natural phenomenon itself. *See* 437 U.S. at 593-95. While petitioner repeatedly invokes this aspect of *Flook* (*e.g.*, Pet. Br. 28, 31), petitioner fails to acknowledge that the *Flook* approach was short-lived.

In *Diehr*, this Court retreated from language in *Flook* and made clear that "[t]he 'novelty' of any element or steps in a process, or even of the process itself, *is of no relevance* in determining whether the subject matter of a claim falls within the § 101 categories of possibly patentable subject matter." *Diehr*, 450 U.S. at 188-89 (emphasis added). Instead, the question "of whether a particular invention is novel is 'wholly apart from whether the invention falls into a category of statutory subject matter.'" *Id.* at 190 (quoting *In re*

*Bergy*, 596 F.2d 952, 961 (C.C.P.A. 1979), *vacated as moot*, 444 U.S. 1028 (1980)).[14] Thus, while a mathematical equation or law of nature "is not patentable in isolation," when incorporated as part of a process that yields a more efficient or useful end, "that process is at the very least not barred at the threshold by § 101." *Id.* at 188; *see also id.* at 187.

Although petitioner would have this Court apply the narrow view of subject matter patentability suggested by language in *Flook*, the Federal Circuit has since recognized—correctly—that *Flook* was "in part superseded" (*Arrhythmia Research Tech., Inc.* v. *Corazonix Corp.*, 958 F.2d 1053, 1057 n.4 (Fed. Cir. 1992)) and "expressly limited" (*Excel*, 172 F.3d at 1356) by *Diehr*. *See* U.S. Cert. Br. 11-15. Thus, a natural phenomenon "in the abstract" does not constitute patentable subject matter, but a claimed invention does meet Section 101's subject matter requirements when the phenomenon "has been reduced to some practical application rendering it 'useful.'" *Excel*, 172 F.3d at 1356-57; *accord Alappat*, 33 F.3d at 1543-44; *State Street*, 149 F.3d 1373.

Like the Federal Circuit, the PTO has adhered to *Diehr*. *See* U.S. Br. 21-22 n.4. Its *Guidelines* instruct patent examiners that a "practical application" of an abstract idea, law of nature, or natural phenomenon is patentable subject matter within the meaning of Section 101 "if the claimed invention

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[14] Although petitioner half-heartedly suggests that the Inventors did not in fact invent the process claimed in the patent (Pet. Br. 4 & n.2), failure to meet the requirements of Sections 102 or 103 "does not affect the determination" whether the patent "recite[s] subject matter which [is] eligible for patent protection under § 101." *Diehr*, 450 U.S. at 190-91 (internal quotation omitted). In any event, respondents established the novelty of the inventive method in the courts below (*see* Resp. C.A. Br. 55-60), and the Federal Circuit affirmed the jury's findings that petitioner had failed to prove anticipation or obviousness. Pet. App. 18a-21a. Petitioner's failure to challenge those factual findings in this Court renders its skewed recitation of history completely immaterial.

physically transforms an article or physical object to a different state or thing, *or* if the claimed invention otherwise produces a useful, concrete, and tangible result." App., *infra*, 5a (emphasis added). Under either of the independently sufficient parts of that test, claim 13 plainly passes the Section 101 threshold.[15]

### i. Claim 13 Entails A Physical Transformation Of Matter

"Transformation and reduction of an article 'to a different state or thing' is the clue to patentability of a process claim that does not include particular machines." *Gottschalk* v. *Benson*, 409 U.S. 63, 70 (1972); *see Cochrane*, 94 U.S. at 788 ("A process is . . . an act, or a series of acts, performed upon the subject-matter to be transformed and reduced to a different state or thing"). In *Diehr*, the Court held that "when a claim containing a mathematical formula implements or applies that formula in a structure or process which, when considered as a whole, is performing a function which the patent laws were designed to protect (*e.g.*, transforming or reducing an article to a different state or thing), then the claim satisfies the requirements of § 101." 450 U.S. at 192.

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[15] Although petitioner maintains (Br. 17, 23-24) that claim 13 involves no "post-solution activity," petitioner's arguments in this regard are misdirected. This Court noted in *Diehr* that "insignificant postsolution activity will not transform an unpatentable principle into a patentable process" (*Diehr*, 450 U.S. at 191-92), but the Court has never held that "post-solution activity" is a prerequisite to patentability. Nor has such a requirement been imposed by the Federal Circuit or the PTO. Indeed, it is not clear that the concept of "post-*solution* activity" has any applicability outside the area of mathematical algorithms (which, by definition, involve a "solution"). *See Flook*, 437 U.S. at 590. Here, because claim 13 undoubtedly meets the subject matter eligibility requirements that this Court *has* imposed, the issue of "post-solution activity" (or, for that matter, "pre-solution activity") simply has no bearing on this case.

The PTO's *Guidelines* recognize that the transformation of matter, in itself, is dispositive of the Section 101 question:

> The examiner first shall review the claim and determine if it provides a transformation or reduction of an article to a different state or thing. If the examiner finds such a transformation or reduction, *the examiner shall end the inquiry and find that the claim meets the statutory requirement of 35 U.S.C. § 101.*

App., *infra*, 14a (emphasis added). Because the invention of claim 13 *requires* the transformation of matter (*i.e.*, blood or other body fluid) in order to diagnose vitamin deficiencies, it is patentable under Section 101.

Petitioner states, without citation, that "Claim 13 recites no such transformative method" because "the assaying step does not direct a practitioner to transform anything." Pet. Br. 27; *see also, e.g.*, CCIA Br. 4 n.4 (asserting that claim 13 "produce[s] no transformation"). That is simply wrong as a matter of both biochemistry and the undisputed evidence.

The assaying step requires "assaying a body fluid for an elevated level of total homocysteine." S.A. 30. It is undisputed that total homocysteine includes at least four species of homocysteine, three of which do not exist in free form. Pet. Br. 2-3 n.1. As the patent itself explains, "[i]n the presence of proteins, . . . homocysteine . . . form[s] complexes with free sulfhydryl groups on the protein molecule; in samples derived from tissues, such protein complexes may tie up most of the . . . homocysteine present." S.A. 13. As a result, "*[r]eduction is required* for release and subsequent assay of protein bound sulfhydryl compounds." *Ibid.* (emphasis added). Or, as an article co-authored by the Inventors explains, "[d]etermination of total Hcy [homocysteine] in plasma/serum *requires the reduction* of the disulfide bond between Hcy and other thiols or albumin." J.A. 262 (emphasis added); *see also* J.A. 247.

Thus, as the Solicitor General has correctly recognized, "the various methods of assaying for total homocysteine that are described in the record entail significant physical or chemical alteration of a sample of blood or other bodily fluid." U.S. Br. 21 n.4. Petitioner has identified no assay method that does *not* involve the transformation of matter, and given the biochemistry involved, no such assay could exist. Thus, petitioner has not carried its burden of proving, by clear and convincing evidence, that claim 13 is invalid because it could be practiced without transforming matter. Nothing more is required under *Diehr* to sustain the patent.[16]

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[16] *Amicus* ACLA wrongly contends (Br. 18) that the transformation that is an integral part of the inventive diagnostic method is insufficient because it is a "means to the end" and "does precisely the reverse" of the patent at issue in *Diehr*. ACLA's novel distinction, however, has no support in the law. Claim 13 sets forth a two-step process, which indisputably cannot be completed without the physical transformation of matter. Contrary to ACLA's theory, *Diehr* says nothing about whether the physical transformation has to be the *final* step in a claimed process; rather, as the PTO *Guidelines* reflect, it holds that if a process performs a physical transformation "function" it passes muster under Section 101. *Diehr*, 450 U.S. at 192. Claim 13 does so. And because claim 13 requires the transformation of matter, petitioner's attempt to liken this case to *Funk Brothers* (Br. 21) is mistaken. In *Funk Brothers*, the Court held that "aggregation of species [of bacteria] fell short of invention within the meaning of the patent statutes" because no transformation had been effected: "The combination of species produces no new bacteria, no change in the six species of bacteria, and no enlargement in the range of their utility." 333 U.S. at 131. The Court has since held that a "nonnaturally occurring" bacterium could be patented because it had "markedly different characteristics from any found in nature." *Chakrabarty*, 447 U.S. at 310. The Inventors' diagnostic method likewise is not to be found in nature, but rather is "a product of human ingenuity" (*id.* at 309) that meets every extant test for subject matter patentability.

### ii. The Method Of Claim 13 Produces A Useful, Tangible, And Concrete Result

Claim 13 also passes the Section 101 threshold for the separately sufficient reason that it produces a useful, tangible, and concrete result. *See* App., *infra*, 15a. Although natural phenomena are not patentable subject matter when they are presented as "merely abstract ideas constituting disembodied concepts or truths that are not 'useful,'" when natural phenomena are incorporated in a claimed invention as part of a process that produces a "useful, concrete, and tangible result," the invention satisfies Section 101. *State Street*, 149 F.3d at 1373, 1375; *Alappat*, 33 F.3d at 1544; *Excel*, 172 F.3d at 1360. The "result" produced under the two-step process set forth in claim 13 is a diagnosis of a condition of the human body, namely the detection of cobalamin or folate deficiencies; this diagnostic result plainly meets the *State Street* factors.

The result produced by the inventive method is clearly "useful" in that it detects a potentially dangerous medical condition and improves the patient's chances of receiving proper treatment. *See, e.g., Arrhythmia Research*, 958 F.2d at 1059-60 (holding that process claims setting forth "a method of analyzing electrocardiograph signals in order to determine a specified heart activity" are directed to statutory subject matter); *id.* at 1066 (Rader, J., concurring) (a method "for detecting the risk of a heart attack" qualifies as statutory subject matter under Section 101). In fact, petitioner concedes, as it must, that claim 13 sets forth a "practical use" by enabling the "detect[ion of] vitamin deficiencies." Pet. Br. 27.

The result produced under claim 13 is also "tangible," which the PTO defines as the opposite of "abstract." App., *infra*, 16a. Unless properly diagnosed, a patient suffering from cobalamin or folate deficiencies may not receive proper supplements to treat his or her condition. Proper medical di-

agnoses have tangible, real-life consequences and are far from "abstract." As with the method patent at issue in *Arrhythmia Research*, claim 13 does not "disclose mere abstract ideas, but a practical and potentially life-saving process." 958 F.2d at 1065-66 (Rader, J., concurring); *see also State Street* 149 F.3d at 1373 (explaining that *Arrhythmia Research* was decided consistently with the "useful, tangible, and concrete result" test); *accord Excel*, 172 F.3d at 1359.

Finally, claim 13 produces a "concrete" result. The PTO defines "concrete" as the opposite of "unrepeatable or unpredictable." App., *infra*, 17a. One of the great benefits of the process set forth in claim 13 is that it yields predictable, repeatable results. S.A. 27-28; J.A. 85, 177. By inventing a method that accurately and repetitively detects the presence or absence of cobalamin or folate deficiencies, the Inventors have added an important diagnostic technique to the arsenal of medical practitioners.

Petitioner has never disputed that claim 13 produces a useful, tangible, and concrete result. Instead, petitioner chooses to ignore the existence of that standard altogether, and clings to language in *Flook* to contend that because the usefulness of the patents at issue in that case "did not save them," it must be true that "specifying one practical use for a scientific correlation does not render a patent claim valid." Pet. Br. 28. That view was rejected in *Diehr*, however, which provided that when a process employing a natural phenomenon results in a practical use, it *does* fall within Section 101. *See Diehr*, 450 U.S. at 188, 192; *State Street*, 149 F.3d at 1375. Petitioner offers no basis for this Court to resurrect the superseded aspects of *Flook* and does not even attempt to square its theory of unpatentability with the rule developed in *Diehr*, applied in the Federal Circuit, and reflected in the PTO's *Guidelines*.[17]

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[17] Petitioner's invocation (Br. 24-25 & n.13) of *In re Grams*, 888 F.2d 835 (Fed. Cir. 1989), does not improve its position.

Nor does petitioner's repeated contention that the patent is invalid because it allegedly precludes doctors from "thinking about" test results (*e.g.*, Pet. Br. 22), provide reason to question the patentability of claim 13. Aside from the fact that a computer could presumably be programmed to perform the correlating step on the results of an assay, the so-called "mental steps test" that petitioner and its amici seek to invoke (Pet. Br. 27 n.14; CCIA Br. 5; AARP Br. 21) was long ago repudiated as an appropriate measure of patentability. *See, e.g.*, App., *infra*, 21a-22a (citing *In re Musgrave*, 431 F.2d 882, 893 (C.C.P.A. 1970)). Thus, even "[i]f all the steps of a claimed process can be carried out in the human mind," the proper inquiry remains "whether the claimed process produces a useful, tangible, and concrete result," as "set forth in *State Street*." *Id.*

In any event, claim 13 is only infringed when the assaying and correlating steps are *both* performed, sequentially, for the purpose of diagnosing vitamin deficiencies. The act of assaying body fluids for total homocysteine for reasons other than diagnosing vitamin deficiencies would not infringe. Nor would the act of correlating alone, or what petitioner calls "thinking about" the relationship between total homocysteine and vitamin deficiencies.[18]

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*Grams* has been expressly disapproved by the Federal Circuit (*Excel*, 172 F.3d at 1360), and no court has cited it since.

[18] Petitioner was found to infringe the patent because petitioner marketed its total homocysteine assays for use in diagnosing vitamin deficiencies via the method of claim 13. J.A. 173, 189-195, 255-256, 257-258, 259-261; *see MGM Studios Inc.* v. *Grokster, Ltd.*, 125 S. Ct. 2764, 2779 (2005) ("The classic case of direct evidence of unlawful purpose occurs when one induces commission of infringement by another, or entices or persuades another to infringe, as by advertising") (internal quotation and citation omitted). Whether other persons or entities infringe the '658 patent is a separate question on which no evidence was presented below. Petitioner attempts to dispute this by noting (Br. 15) that one of the

### b. The Claimed Invention Does Not "Preempt" Public Use Of Any Natural Phenomenon

As just demonstrated, claim 13 recites a practical application of the correlation between elevated total homocysteine and cobalamin and folate deficiencies discovered by the Inventors. The only remaining question on patentability is whether claim 13, as construed by the lower courts, is so sweeping as to "preempt" public use of that correlation. *Diehr*, 450 U.S. at 191; *see* App., *infra*, 15a. A patent sweeps too broadly if it comprises every "substantial practical application" of a natural phenomenon, because it "in practical effect would be a patent on the [phenomenon] itself." *Benson*, 409 U.S. at 71-72.

Respondents do not seek, and the '658 patent does not claim, a monopoly on the correlation between total homocysteine and vitamin deficiencies. Rather, the Inventors have patented a particular *application* of that correlation, when used as a sequential step in a diagnostic method. In this regard, the Inventors here are analogous to the patentees in *Diehr*: "Their process admittedly employs a well-known mathematical equation, but they do not seek to pre-empt use of that equation. Rather, they seek only to foreclose from others the use of that equation in conjunction with all of the other steps in their claimed process." 450 U.S. at 187.[19]

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Inventors "testified that it would be malpractice for a doctor to receive a total homocysteine assay without determining cobalamin/folate deficiency." Pet. App. 14a. What the Inventor actually said was that it would be malpractice to "fail to treat in response to [a] high homocysteine level." J.A. 106. She was not asked whether the purpose of the hypothetical assay was to detect cobalamin and folate deficiencies or, for example, to instead diagnose an inherited enzyme defect. *See* note 13, *supra*.

[19] For this reason, petitioner errs in attempting to equate the '658 patent with Samuel Morse's effort to monopolize "electro-

The government asserts, incorrectly, that "claim 13 appears to cover all substantial practical applications of the natural phenomenon." U.S. Br. 24. That assertion rests entirely on the jury's finding in connection with the contributory infringement claim (which was not considered by the court of appeals) "that no *substantial* non-infringing uses *of the total homocysteine assays* had been proven on the trial record." *Id.* at 23 (emphases added).[20] But the "natural phenomenon" at issue in this case is *not* the assay performed by petitioner, but rather the *correlation* between total homocysteine and vitamin deficiencies—as the government elsewhere acknowledges. *Id.* at 19. The jury made *no* finding that claim 13 covers substantially all practical applications of that correlation.

Contrary to the government's suggestion, there is no reason to "vacate and remand for further proceedings to determine whether all substantial practical applications of the correlation are claimed by the patent." U.S. Br. 26-27. As the government acknowledges, *petitioner* bears the burden of proving that no such applications exist. *Id.* at 24. Yet petitioner offers only the bare assertion that "[t]he scientific

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magnetism." Pet. Br. 35 (quoting *O'Reilly* v. *Morse*, 56 U.S. (15 How.) 62, 112 (1853)). Whereas Morse claimed all uses of a natural phenomenon, the '658 patent claims just one. The more apt analogy is Alexander Graham Bell's telephone patent. *Dolbear* v. *Am. Bell Tel. Co.*, 126 U.S. 1, 535 (1888) ("It may be that electricity cannot be used at all for the transmission of speech except in the way Bell has discovered, and that therefore, practically, his patent gives him its exclusive use for that purpose, but that does not make his claim one for the use of electricity distinct from the particular process with which it is connected in his patent").

[20] Although the jury found that there were no substantial non-infringing uses for the assays performed *by petitioner* (a for-profit provider of test results), it made no such finding as to total homocysteine assays performed by others for a different purpose (such as detecting an inherited enzyme defect).

principle that elevated homocysteine is associated with vitamin deficiencies is substantially covered by Claim 13" (Pet. Br. 28), citing *nothing* in the record—let alone the clear and convincing evidence that would be required to prevail. If the record were entirely silent, then the statutory presumption of validity would require affirmance of the judgment. This Court does not sit to order remands for consideration of issues that the petitioner failed to raise at any previous stage of the litigation. *E.g.*, *Pasquantino* v. *United States*, 125 S. Ct. 1766, 1781 n.14 (2005); *Yee*, 503 U.S. at 533.[21]

In any event, the record in fact discloses several non-infringing uses of the correlation. One such use is suggested by a study where the authors recommend cobalamin and folate supplements for all men over 45, and all women over 55, in order to reduce and prevent elevated total homocysteine levels (which are associated with heart disease and other illnesses), *without* first assaying total homocysteine in any patient. J.A. 107-108, 204-205; C.A. App. 4687-4688, 8711-8717. The authors estimated that this treatment strategy would save hundreds of thousands of lives, and billions

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[21] Although petitioner now asks this Court to hold the patent invalid and "reverse the judgment against [petitioner] in its entirety" (Pet. Br. 19), an order directing entry of judgment against respondents is obviously precluded by the Seventh Amendment because no jury has found the *factual* predicates to a Section 101 defense. *Dimick* v. *Schiedt*, 293 U.S. 474, 486-87 (1935). Moreover, on the record in this case it would be inconceivable for the Court to remand for further proceedings. *Unitherm*, slip op. 7 ("This Court's observations about the necessity of a postverdict motion under Rule 50(b), and the benefits of the district court's input at that stage, apply with equal force whether a party is seeking judgment as a matter of law or simply a new trial"). Indeed, petitioner's request to be placed in a better position than it would have occupied had it complied with all applicable substantive and procedural rules speaks volumes not only of the weakness of its position but also of its misunderstanding of the role of this Court in our judicial system.

of dollars, over a ten-year period. J.A. 205. In another study, the authors determined that the toxicity of a chemotherapy treatment appeared related to patients' levels of total homocysteine. Aware of the correlation discovered by these Inventors, the authors recommended administering supplemental cobalamin and folate, thereby lowering the toxicity of an important drug that "has demonstrated promising clinical activity in a wide variety of solid tumors, including non-small cell lung, breast, mesothelioma, colorectal, pancreatic, gastric, bladder, cervix, and head and neck." C.A. App. 9763. Cancer patients who receive this drug today receive it in conjunction with supplemental cobalamin and folate as recommended by these authors. C.A. App. 4713-4714, 9763-9778.[22] Treating patients with vitamin supplements to reduce the risk of heart attack or the toxicity of a cancer drug, without first assaying for total homocysteine, constitute uses of the correlation at issue that do not infringe the patent.

Moreover, a number of other non-infringing uses of the correlation discovered by the Inventors appear in the medical literature, which is readily available to persons skilled in the art. As just one example, a recent study posited that elevated total homocysteine could play a role in the incidence of bone fractures sustained by stroke victims. To test this hypothesis, half of a control group were given placebos while the other half received cobalamin and folate supplements; the patients who received vitamin supplements suffered far fewer fractures than the ones who did not. *See* Sato, *et al.*, *Effect of Folate and Mecobalamin on Hip Fractures in Patients with Stroke*, 293 J. Am. Med. Assoc. 1082 (2005). As a practitioner's resource recently summarized this study, "it is reason-

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[22] Since the record in this case was closed, the FDA has approved this drug for the treatment of mesothelioma, an asbestos-related cancer, as well as to treat the most common form of lung cancer. *See* Rollins & Lindley, *Pemetrexed: A Multitargeted Antifolate*, 27 Clinical Therapeutics 1343 (2005).

able to give these inexpensive and safe vitamins [$B_{12}$ and folate] to patients with stroke." American College of Physicians Journal Club, *Folate plus vitamin $B_{12}$ reduced hip fractures in patients with poststroke hemiplegia*, Vol. 143 No. 2 (Sept./Oct. 2005). Treating stroke victims with vitamin supplements to reduce fractures, without first assaying for total homocysteine, is yet another use of the correlation discovered by the Inventors that does not infringe the '658 patent.

Many more substantial practical applications of the correlation that do not infringe claim 13 are undoubtedly being practiced currently, or could be developed in the future, but these examples will suffice to show that petitioner has not carried *its* burden of proving, by clear and convincing evidence, that such uses are absent and the patent is therefore invalid. Rather, the examples confirm claim 13's validity under the government's own analysis. U.S. Br. 24 n.5, 26. The patent is not "preemptive" within the meaning of *Diehr* and *Benson*.

### C. The Sea Change Sought By Petitioner Would Be Unnecessarily Disruptive To The Patent System

As demonstrated above, claim 13 of the '658 patent meets the subject matter eligibility requirements of current law, as enunciated by this Court (in *Diehr*), the Federal Circuit (in *State Street*, *Excel*, and *Alappat*), and the PTO (in its interim *Guidelines*). Thus, in order to grant petitioner any relief on the subject matter patentability argument that it has raised for the first time in this Court, the Court would have to revisit *Diehr*, overturn settled Federal Circuit precedent, and disapprove the standards by which the PTO has reviewed and issued tens of thousands of patents.

Petitioner seems intent on obscuring from the Court just how radical its position is. By clinging to *Flook* as if it had been left untouched by *Diehr*, failing even to cite the principal Federal Circuit decisions, and ignoring the PTO's *Guide-*

*lines*, petitioner has chosen to mount a sneak attack on the governing law. But if petitioner were to obtain a decision from this Court questioning the validity of the '658 patent on the ground that it claims non-patentable subject matter, 25 years of consistently applied patent law would come undone.

### 1. Vacatur Would Call Into Question Thousands Of Issued Patents

"Since this Court decided *Diehr* almost 25 years ago, PTO has generally followed the Federal Circuit's understanding that *Diehr* substantially limited *Flook*, and has issued numerous patents based on that understanding—including patents on medical diagnostic methods, other types of diagnostic and testing procedures, and computer-related processes." U.S. Cert. Br. 14. If this Court were to accept petitioner's invitation and change the law of subject matter patentability, the validity of some or all of those patents would be thrown into doubt. "A decision overturning PTO's approach could call into question a substantial number of patent claims and undermine the settled expectations of numerous participants in technology-based industries." *Ibid.*; *cf. Warner-Jenkinson Co.* v. *Hilton Davis Chem. Co.*, 520 U.S. 17, 32 n.6 (1997) ("To change so substantially the rules of the game now could very well subvert the various balances the PTO sought to strike when issuing the numerous patents which have not yet expired and which would be affected by our decision").[23]

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[23] While petitioner seeks to minimize the impact of a decision in its favor, its *amici* are more straightforward. They recognize that such a decision would further their agenda of abolishing all past, present, and future patents on business methods, software, gene sequences, or medical procedures. *E.g.*, CCIA Br. 2, 6; Financial Services Industry Br. 7-8, 14-17; Affymetrix, Inc. Br. 3-4, 15-16; AARP Br. 2, 9. The wholesale challenge to *State Street* and its progeny mounted by these *amici* should await resolution in

1. A decision questioning the validity of claim 13 of the '658 patent would especially call into question hundreds or thousands of patents on medical diagnostic methods, which frequently recite the sequential steps of assaying (or determining) the amount of a particular substance of the body and correlating the determined amount with a disease. Claim 1 of U.S. Patent No. 6,811,993, for example, claims a three-step diagnostic method of "determining the level of [Protein Kinase C] activity in monocytes of the subject; optionally comparing the level of PKC activity in monocytes of the subject with a standard; and correlating the level of PKC activity with the extent, stage, or severity of the cardiovascular complication of diabetes." Because many diseases are indicated by "markers"—proteins, enzymes, amino acids, or other substances that change (in quantity or behavior) in the presence of the disease—patents on useful diagnostic processes that employ such markers are in fact common. *See* U.S. Patent Nos. 6,461,831 (Background and Written Description) (discussion of this issue in the context of Alzheimer's disease); 6,929,918 (Background and Written Description) (same in the context of prostate cancer).[24]

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a case in which the issue is actually presented by a party. *See Bell* v. *Wolfish*, 441 U.S. 520, 531-32 n.13 (1979).

[24] Although petitioner maintains (Br. 19) that "[c]orrelations are elemental tools of all science, and as such are free to all and patentable by none," a search of the PTO's database indicates that approximately 20,000 patents have issued since 1976 with "correlate" (or a variant thereof) as a claim limitation. The government clearly does not share petitioner's view that correlations are *per se* unpatentable. *See also, e.g.*, U.S. Patent Nos. 6,004,528 (Claim 10) ("method for aiding in the diagnosis of cancer"); 6,962,793 (Claim 1) ("method for diagnosing Alzheimer's disease"); U.S. Patent No. 6,979,533 (Claim 1) ("method for detecting receptivity of the endometrium of a mammal to embryo implantation"); U.S. Patent No. 4,836,218 (Claim 1) (method for detecting temporomandibular joint disorders). *Amicus* ACLA expresses concern (Br.

The havoc caused by a decision questioning the validity of the '658 patent would extend far beyond the realm of medical diagnoses to every patented invention that incorporates a natural relationship (including most drugs, many medical devices, and a host of computer software and hardware applications). For example, on petitioner's theory Bell's process for communicating information over the telephone would have been unpatentable, because it was merely a practical application of the natural phenomenon that electricity can be used to propagate sound waves over a wire. *But see* note 19, *supra*. And pharmaceutical companies could not patent methods of treating conditions such as depression, Alzheimer's, or heart disease with drugs, since they have merely discovered that certain chemicals interact with the human body in ways directed by chemistry and patented practical applications of such discovered interactions. *Cf. Diehr*, 450 U.S. at 189 n.12 ("To accept the analysis proffered by the petitioner would, if carried to its extreme, make all inventions unpatentable because all inventions can be reduced to underlying principles of nature which, once known, make their implementation obvious").

2. The PTO, and the Federal Circuit, have implemented *Diehr* with a workable set of principles of subject matter eligibility that have guided the issuance and review of patents for the past quarter-century. If those standards are flawed such that they ought to be changed, petitioner and its *amici* have at least three avenues of redress.

First, the PTO itself has invited public comment on the standards of subject matter patentability. 70 Fed. Reg. at 75,452; *see Warner-Jenkinson*, 520 U.S. at 33-34 (noting the

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11-13) that a method of diagnosing prostate cancer using antibody assays could be patented. In fact, numerous patents have issued on such diagnostic methods. In addition to Claim 7 of the '918 patent cited in the text above, *see, e.g.*, U.S. Patent No. 6,482,599 (Claim 35).

"primacy of the PTO in ensuring that the claims allowed cover only subject matter that is properly patentable"). Its final guidance, which presumably will be entitled to deference under *Chevron U.S.A. Inc.* v. *Natural Resources Defense Council, Inc.*, 467 U.S. 837, 842-45 (1984), could materially assist in this Court's ultimate review of the issue.

Second, Congress can always step in to change the Patent Act. Indeed, a comprehensive patent reform bill is pending at this moment. *See* "Patent Reform Act of 2005," H.R. 2795, 109th Cong., 1st Sess. (introduced June 8, 2005); *cf. Warner-Jenkinson*, 520 U.S. at 28 ("Congress can legislate the doctrine of equivalents out of existence any time it chooses"). Its decision for the past quarter-century not to override *Diehr* suggests an acceptance of it. *See J.E.M. Ag Supply, Inc.* v. *Pioneer Hi-Bred Int'l, Inc.*, 534 U.S. 124, 145-46 (2001) ("As in *Chakrabarty*, we decline to narrow the reach of § 101 where Congress has given us no indication that it intends this result"). This is especially so here, where Congress in the interim has responded to complaints about the patentability of other types of inventions not by restricting patentable subject matter, but by limiting remedies or adding defenses for those inventions. *See* 35 U.S.C. § 287(c) (restricting remedies for surgical procedure patents); 35 U.S.C. § 273(a)(3) (adding prior user defense against business method patents).

And third, the Federal Circuit, established by Congress as the expert court in the patent area, should at least be given the first opportunity to consider these issues. *See Warner-Jenkinson*, 520 U.S. at 40 (noting the Federal Circuit's "sound judgment" in the patent area of its "special expertise").

Petitioner wants to bypass all of these avenues and asks this Court to rule, in the first instance and without the benefit of a full record, that an issued patent, imbued with the statutory presumption of validity, is invalid for failing to claim

patentable subject matter notwithstanding the fact that claim 13 passes all extant requirements for subject matter eligibility. As the Solicitor General has explained, "if this Court were to consider reevaluating almost a quarter-century of administrative practice and lower court jurisprudence, it should do so based on a full record in a case where the issue was properly raised, litigated, and decided below." U.S. Cert. Br. 19. This is not that case.

### 2. Affirmance (Or Dismissal) Would Have No Adverse Consequences In The Context Of This Case

Petitioner and its *amici* predict dire consequences for public health if the '658 patent stands, going so far as to assert that a decision for respondents would "depriv[e] patients of needed medical services." Pet. Br. 30. At best, these are policy arguments better addressed to the political branches; at worst, they are nothing but scare tactics.

Petitioner is a for-profit company that is seeking to maximize its revenue by avoiding the royalties due respondents under the licensing agreement that petitioner previously entered into. There is not a shred of evidence in the record that petitioner's payment of royalties to respondents, or the ultimate cost of the homocysteine assays performed by petitioner, have ever affected a single doctor's treatment decision. There is absolutely no evidence that, if the judgment below were affirmed (or the writ dismissed), any patient's care, or the cost of that care, would change in any way.[25]

Patented inventions are common in the field of medicine. In addition to diagnostic methods, a physician may employ

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[25] Nor is there any evidence that any doctor will be held liable for patent infringement. As respondents explained in their brief in opposition (at 9), and petitioner has not contested, "[n]ot a single physician has been enjoined, sued or even threatened with suit, and none will be."

diagnostic machinery (such as an MRI machine), medical devices (such as implants or prosthetics), and of course a wide range of pharmaceuticals, all of which may be covered by one or more patents. For this reason, AARP's assertion (Br. 9) that claim 13 "would prohibit physicians from practicing good medicine without a patent license" proves far too much. "Good medicine" may in fact require physicians to practice patented inventions on a daily basis—by engaging in patented diagnostic methods, by using patented devices, or by prescribing patented pharmaceuticals. Although the patent regime may increase (or decrease) the cost of patient care, Congress has made the policy judgment that the benefits of increased innovation provided by the patent system justify any distortions that patents might introduce into the healthcare delivery system.

Indeed, this case proves the soundness of Congress's policy judgment: The patent system helped motivate these Inventors and their universities and agents to conceive of, develop and commercialize an invention that has saved thousands of lives and millions of dollars in health care costs by allowing for prompt and accurate diagnoses of a serious but easily treated condition. *Cf. Festo Corp.* v. *Shoketsu Kinzoku Kogyo Kabushiki Co., Ltd.*, 535 U.S. 722, 739 (2002) ("Fundamental alterations in these rules risk destroying the legitimate expectations of inventors in their property"). Indeed, over ten million homocysteine assays are now performed each year to detect cobalamin and folate deficiencies.

As Justice Breyer has recognized, this area of the law is a particularly thorny one. Breyer, *supra*, 28 J. L. Med. & Ethics at 27 ("Should it matter if the more apt description of the scientist's work is the 'discovery' of how a portion of the body functions, rather than the 'invention' of how to use a part of that body to perform a useful, say, diagnostic task? This latter question will sometimes seem unanswerable."). For now, the PTO and the Federal Circuit have found *Diehr* a workable guide, and Congress has not seen fit to provide oth-

erwise. If the law is to be changed, it should be in a case in which the issues are pleaded, litigated, and decided at all levels, with full input from all potentially affected constituencies—*not* in this case, where the question of subject matter patentability has been raised by petitioner only at the last stage of the proceedings, and on a spare if not empty record.

## CONCLUSION

The judgment of the court of appeals should be affirmed. In the alternative, the writ of certiorari should be dismissed.

Respectfully submitted.

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Title 35 U.S.C. § 100 provides in pertinent part:

**§ 100. Definitions**

When used in this title unless the context otherwise indicates—

(a) The term "invention" means invention or discovery.

(b) The term "process" means process, art or method, and includes a new use of a known process, machine, manufacture, composition of matter, or material.

* * * *

Title 35 U.S.C. § 102 provides in pertinent part:

**§ 102. Conditions for patentability; novelty and loss of right to patent**

A person shall be entitled to a patent unless—

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent, or

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States, or

* * * *

(f) he did not himself invent the subject matter sought to be patented[.]

* * * *

Title 35 U.S.C. § 103 provides in pertinent part:

**§ 103. Conditions for patentability; non-obvious subject matter**

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

* * * *

Title 35 U.S.C. § 282 provides in pertinent part:

**§ 282. Presumption of validity; defenses**

A patent shall be presumed valid. Each claim of a patent (whether in independent, dependent, or multiple dependent form) shall be presumed valid independently of the validity of other claims * * *. The burden of establishing invalidity of a patent or any claim thereof shall rest on the party asserting such invalidity.

The following shall be defenses in any action involving the validity or infringement of a patent and shall be pleaded:

* * * *

(2) Invalidity of the patent or any claim in suit on any ground specified in part II of this title [§§ 100 *et seq.*] as a condition for patentability,

(3) Invalidity of the patent or any claim in suit for failure to comply with any requirement of sections 112 or 251 of this title, [or]

(4) Any other fact or act made a defense by this title.

* * * *

Federal Rule of Civil Procedure 8 provides in pertinent part:

**Rule 8. General Rules of Pleading**

* * * *

(c) Affirmative Defenses. In pleading to a preceding pleading, a party shall set forth affirmatively accord and satisfaction, arbitration and award, assumption of risk, contributory negligence, discharge in bankruptcy, duress, estoppel, failure of consideration, fraud, illegality, injury by fellow servant, laches, license, payment, release, res judicata, statute of frauds, statute of limitations, waiver, and any other matter constituting an avoidance or affirmative defense. When a party has mistakenly designated a defense as a counterclaim or a counterclaim as a defense, the court on terms, if justice so requires, shall treat the pleading as if there had been a proper designation.

* * * *

Patent Act of 1870, ch. 230, § 61, 16 Stat. 198, 208 provides in pertinent part:

**Sec. 61**. *And be it further enacted,* That in any action for infringement the defendant may plead the general issue, and having given notice in writing to the plaintiff or his attorney, thirty days before, may prove on trial any one or more of the following special matters: —

First. That for the purpose of deceiving the public the description and specification filed by the patentee in the patent office was made to contain less than the whole truth relative to his invention or discovery, or more than is necessary to produce the desired effect; or,

Second. That he had surreptitiously or unjustly obtained the patent for that which was in fact invented by another,

who was using reasonable diligence in adapting and perfecting the same; or,

Third. That it had been patented or described in some printed publication prior to his supposed invention or discovery thereof; or,

Fourth. That he was not the original and first inventor or discovered of any material and substantial part of the thing patented; or,

Fifth. That it had been in public use or on sale in this country, for more than two years before his application for a patent, or had been abandoned to the public.

* * * *

* * * *

The *Official Gazette of the United States Patent and Trademark Office*, Vol. 1300, No. 4, pp. 142 *ff.* (November 22, 2005), provides in pertinent part:

**Interim Guidelines for Examination of Patent Applications for Patent Subject Matter Eligibility**

* * * *

The principal objective of these guidelines is to assist examiners in determining, on a case-by-case basis, whether a claimed invention falls within a judicial exception to statutory subject matter (i.e., is nothing more than an abstract idea, law of nature, or natural phenomenon), or whether it is a practical application of a judicial exception to statutory subject matter. The guidelines explain that a practical application of a 35 U.S.C. § 101 judicial exception is claimed if the claimed invention physically transforms an article or physical object to a different state or thing, or if the claimed invention otherwise produces a useful, concrete, and tangible result.

## I. INTRODUCTION

These Examination Guidelines ("Guidelines") are based on the USPTO's current understanding of the law and are believed to be fully consistent with binding precedent of the Supreme Court, the Federal Circuit and the Federal Circuit's predecessor courts.

These Guidelines do not constitute substantive rulemaking and hence do not have the force and effect of law. These Guidelines have been designed to assist USPTO personnel in analyzing claimed subject matter for compliance with substantive law. Rejections will be based upon the substantive law and it is these rejections which are appealable. Conse-

quently, any failure by USPTO personnel to follow the Guidelines is neither appealable nor petitionable.

The Guidelines set forth the procedures USPTO personnel will follow when examining applications. USPTO personnel are to rely on these Guidelines in the event of any inconsistent treatment of issues between these Guidelines and any earlier provided guidance from the USPTO.

* * * *

Annex I which appears at the end of this section includes a flow chart of the process USPTO personnel should follow.

* * * *

## IV. DETERMINE WHETHER THE CLAIMED INVENTION COMPLIES WITH THE SUBJECT MATTER ELIGIBILITY REQUIREMENT OF 35 U.S.C. § 101

### A. Consider the Breadth of 35 U.S.C. § 101 Under Controlling Law

Section 101 of title 35, United States Code, provides:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

As the Supreme Court held, Congress chose the expansive language of 35 U.S.C. § 101 so as to include "anything under the sun that is made by man." *Diamond v. Chakrabarty*, 447 U.S. 303, 308-09, 206 USPQ 193, 197 (1980). In *Chakrabarty*, 447 U.S. at 308-309, 206 USPQ at 197, the court stated:

> In choosing such expansive terms as "manufacture" and "composition of matter," modified by the

comprehensive "any," Congress plainly contemplated that the patent laws would be given wide scope. The relevant legislative history also supports a broad construction. The Patent Act of 1793, authored by Thomas Jefferson, defined statutory subject matter as "any new and useful art, machine, manufacture, or composition of matter, or any new or useful improvement [thereof]." Act of Feb. 21, 1793, ch. 11, § 1, 1 Stat. 318. The Act embodied Jefferson's philosophy that "ingenuity should receive a liberal encouragement." V Writings of Thomas Jefferson, at 75-76. See *Graham v. John Deere Co.*, 383 U.S. 1, 7-10 (148 USPQ 459, 462-464) (1966). Subsequent patent statutes in 1836, 1870, and 1874 employed this same broad language. In 1952, when the patent laws were recodified, Congress replaced the word "art" with "process," but otherwise left Jefferson's language intact. The Committee Reports accompanying the 1952 Act inform us that Congress intended statutory subject matter to "include anything under the sun that is made by man." S. Rep. No. 1979, 82d Cong., 2d Sess., 5 (1952); H.R. Rep. No. 1923, 82d Cong., 2d Sess., 6 (1952). [Footnote omitted]

This perspective has been embraced by the Federal Circuit:

> The plain and unambiguous meaning of section 101 is that any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may be patented if it meets the requirements for patentability set forth in Title 35, such as those found in sections 102, 103, and 112. The use of the expansive term "any" in section 101 represents Congress's intent not to place any restrictions on the subject matter for which a patent may be obtained beyond those specifically

> recited in section 101 and the other parts of Title 35 . . . Thus, it is improper to read into section 101 limitations as to the subject matter that may be patented where the legislative history does not indicate that Congress clearly intended such limitations.

[*In re*] *Alappat*, 33 F.3d [1526,] 1542, 31 USPQ2d [1545,] 1556 [(Fed. Cir. 1994) (en banc)].

35 U.S.C. § 101 defines four categories of inventions that Congress deemed to be the appropriate subject matter of a patent: processes, machines, manufactures and compositions of matter. The latter three categories define "things" or "products" while the first category defines "actions" (i.e., inventions that consist of a series of steps or acts to be performed). See 35 U.S.C. 100(b) ("The term 'process' means process, art, or method, and includes a new use of a known process, machine, manufacture, composition of matter, or material.").

Federal courts have held that 35 U.S.C. § 101 does have certain limits. First, the phrase "anything under the sun that is made by man" is limited by the text of 35 U.S.C. § 101, meaning that one may only patent something that is a machine, manufacture, composition of matter or a process. *See, e.g., Alappat*, 33 F.3d at 1542, 31 USPQ2d at 1556; *In re Warmerdam*, 33 F.3d 1354, 1358, 31 USPQ2d 1754, 1757 (Fed. Cir. 1994). Second, 35 U.S.C. § 101 requires that the subject matter sought to be patented be a "useful" invention. Accordingly, a complete definition of the scope of 35 U.S.C. § 101, reflecting Congressional intent, is that any new and useful process, machine, manufacture or composition of matter under the sun that is made by man is the proper subject matter of a patent.

The subject matter courts have found to be outside of, or exceptions to, the four statutory categories of invention is limited to abstract ideas, laws of nature and natural phenomena. While this is easily stated, determining whether an ap-

plicant is seeking to patent an abstract idea, a law of nature or a natural phenomenon has proven to be challenging. These three exclusions recognize that subject matter that is not a *practical application or use* of an idea, a law of nature or a natural phenomenon is not patentable. *See, e.g., Rubber-Tip Pencil Co. v. Howard*, 87 U.S. (20 Wall.) 498, 507 (1874) ("idea of itself is not patentable, but a new device by which it may be made practically useful is"); *Mackay Radio & Telegraph Co. v. Radio Corp. of America*, 306 U.S. 86, 94, 40 USPQ 199, 202 (1939) ("While a scientific truth, or the mathematical expression of it, is not patentable invention, a novel and useful structure created with the aid of knowledge of scientific truth may be."); *Warmerdam*, 33 F.3d at 1360, 31 USPQ2d at 1759 ("steps of 'locating' a medial axis, and 'creating' a bubble hierarchy . . . describe nothing more than the manipulation of basic mathematical constructs, the paradigmatic 'abstract idea'").

The courts have also held that a claim may not preempt ideas, laws of nature or natural phenomena. The concern over preemption was expressed as early as 1852. *See Le Roy v. Tatham*, 55 U.S. (14 How.) 156, 175 (1852) ("A principle, in the abstract, is a fundamental truth; an original cause; a motive; these cannot be patented, as no one can claim in either of them an exclusive right."); *Funk Bros. Seed Co. v. Kalo Inoculant Co.*, 333 U.S. 127, 132, 76 USPQ 280, 282 (1948) (combination of six species of bacteria held to be non-statutory subject matter). Accordingly, one may not patent every "substantial practical application" of an idea, law of nature or natural phenomena because such a patent "in practical effect be a patent on the [idea, law of nature or natural phenomena] itself." *Gottschalk v. Benson*, 409 U.S. 63, 71-72, 175 USPQ 673, 676 (1972).

## B. Determine Whether the Claimed Invention Falls Within An Enumerated Statutory Category

To properly determine whether a claimed invention complies with the statutory invention requirements of 35 U.S.C. § 101, USPTO personnel must first identify whether the claim falls within at least one of the four enumerated categories of patentable subject matter recited in section 101 (process, machine, manufacture or composition of matter).

In many instances it is clear within which of the enumerated categories a claimed invention falls. Even if the characterization of the claimed invention is not clear, this is usually not an issue that will preclude making an accurate and correct assessment with respect to the section 101 analysis. The scope of 35 U.S.C. § 101 is the same regardless of the form or category of invention in which a particular claim is drafted. *AT&T* [*Corp. v. Excel Communications, Inc.*], 172 F.3d [1352,] 1357, 50 USPQ2d [1447,] 1451 [(Fed. Cir. 1999)]. *See also State Street* [*Bank & Trust Co. v. Signature Financial Group, Inc.*], 149 F.3d [1368,] 1375, 47 USPQ2d [1596,] 1602 [(Fed. Cir. 1998),] wherein the Federal Circuit explained

> The question of whether a claim encompasses statutory subject matter should not focus on *which* of the four categories of subject matter a claim is directed to—process, machine, manufacture, or composition of matter—[provided the subject matter falls into at least one category of statutory subject matter] but rather on the essential characteristics of the subject matter, in particular, its practical utility.

For example, a claimed invention may be a combination of devices that appear to be directed to a machine and one or more steps of the functions performed by the machine. Such instances of mixed attributes, although potentially confusing as to which category of patentable subject matter it belongs in, does not affect the analysis to be performed by the exam-

iner. Note that an apparatus claim with process steps is not classified as a "hybrid" claim; instead, it is simply an apparatus claim including functional limitations. *See, e.g., R.A.C.C. Indus. v. Stun-Tech, Inc.*, 178 F.3d 1309 (Fed. Cir. 1998) (unpublished).

The burden is on the USPTO to set forth a *prima facie* case of unpatentability. Therefore if the examiner determines that it is more likely than not that the claimed subject matter falls outside all of the statutory categories, the examiner must provide an explanation. For example, a claim reciting only a musical composition, literary work, compilation of data, or legal document (e.g., an insurance policy) *per se* does not appear to be a process, machine, manufacture, or composition of matter. If the examiner can establish a *prima facie* case that a claim does not fall into a statutory category, that does not preclude complete examination of the application for satisfaction of all other conditions of patentability. The examiner must further continue with the statutory subject matter analysis as set forth below. Also, the examiner must still examine the claims for compliance with 35 U.S.C. §§ 102, 103, and 112.

If the invention as set forth in the written description is statutory, but the claims define subject matter that is not, the deficiency can be corrected by an appropriate amendment of the claims. In such a case, USPTO personnel should reject the claims drawn to nonstatutory subject matter under 35 U.S.C. § 101, but identify the features of the invention that would render the claimed subject matter statutory if recited in the claim.

## C. Determine Whether the Claimed Invention Falls Within § 101 Judicial Exceptions—Laws of Nature, Natural Phenomena and Abstract Ideas

Determining whether the claim falls within one of the four enumerated categories of patentable subject matter recited in 35 U.S.C. § 101 (process, machine, manufacture or composition of matter) does not end the analysis because claims directed to nothing more than abstract ideas (such as mathematical algorithms), natural phenomena, and laws of nature are not eligible and therefore are excluded from patent protection. [*Diamond v.*] *Diehr*, 450 U.S. [175,] 185, 209 USPQ [1,] 7 [(1981)]; accord, e.g., *Chakrabarty*, 447 U.S. at 309, 206 USPQ at 197; *Parker v. Flook*, 437 U.S. 584, 589, 198 USPQ 193, 197 (1978); *Benson*, 409 U.S. at 67-68 , 175 USPQ at 675; *Funk*, 333 U.S. at 130, 76 USPQ at 281. "A principle, in the abstract, is a fundamental truth; an original cause; a motive; these cannot be patented, as no one can claim in either of them an exclusive right." *Le Roy*, 55 U.S. (14 How.) at 175. Instead, such "manifestations of laws of nature" are "part of the storehouse of knowledge," "free to all men and reserved exclusively to none." *Funk*, 333 U.S. at 130, 76 USPQ at 281.

Thus, "a new mineral discovered in the earth or a new plant found in the wild is not patentable subject matter" under Section 101. *Chakrabarty*, 447 U.S. at 309, 206 USPQ at 197. "Likewise, Einstein could not patent his celebrated law that $E=mc^2$; nor could Newton have patented the law of gravity." *Ibid.* Nor can one patent "a novel and useful mathematical formula," *Flook*, 437 U.S. at 585, 198 USPQ at 195; electromagnetism or steam power, *O'Reilly v. Morse*, 56 U.S. (15 How.) 62, 113-114 (1853); or "[t]he qualities of * * * bacteria, * * * the heat of the sun, electricity, or the qualities of metals," *Funk*, 333 U.S. at 130, 76 USPQ at 281; see *Le Roy*, 55 U.S. (14 How.) at 175.

While abstract ideas, natural phenomena, and laws of nature are not eligible for patenting, methods and products employing abstract ideas, natural phenomena, and laws of nature to perform a real-world function may well be. In evaluating whether a claim meets the requirements of section 101, the claim must be considered as a whole to determine whether it is for a particular *application* of an abstract idea, natural phenomenon, or law of nature, rather than for the abstract idea, natural phenomenon, or law of nature itself.

### 1. Determine Whether the Claimed Invention Covers Either a § 101 Judicial Exception or a Practical Application of a § 101 Judicial Exception

An examiner must ascertain the scope of the claim to determine whether it covers either a § 101 judicial exception or a practical application of a § 101 judicial exception. The conclusion that a particular claim *includes* a § 101 judicial exception does not end the inquiry because "[i]t is now commonplace that an *application* of a law of nature or mathematical formula to a known structure or process may well be deserving of patent protection." *Diehr*, 450 U.S. at 187, 209 USPQ at 8 (emphasis in original); accord *Flook*, 437 U.S. at 590, 198 USPQ at 197; *Benson*, 409 U.S. at 67, 175 USPQ at 675. Thus, "[w]hile a scientific truth, or the mathematical expression of it, is not a patentable invention, a novel and useful structure created with the aid of knowledge of scientific truth may be." *Diehr*, 450 U.S. at 188, 209 USPQ at 8-9 (quoting *Mackay*, 306 U.S. at 94); see also *Corning v. Burden*, 56 U.S. (15 How.) 252, 268, 14 L.Ed. 683 (1854) ("It is for the discovery or invention of some practical method or means of producing a beneficial result or effect, that a patent is granted . . .").

## 2. Determine Whether the Claimed Invention is a Practical Application of an Abstract Idea, Law of Nature, or Natural Phenomenon (§ 101 Judicial Exceptions)

For claims including such excluded subject matter to be eligible, the claim must be for a *practical application* of the abstract idea, law of nature, or natural phenomenon. *Diehr*, 450 U.S. at 187, 209 USPQ at 8 ("*application* of a law of nature or mathematical formula to a known structure or process may well be deserving of patent protection."); *Benson*, 409 U.S. at 71, 175 USPQ at 676 (rejecting formula claim because it "has no substantial practical application").

To satisfy section 101 requirements, the claim must be for a practical application of the § 101 judicial exception, which can be identified in various ways:

- The claimed invention "transforms" an article or physical object to a different state or thing.
- The claimed invention otherwise produces a useful, concrete and tangible result, based on the factors discussed below.

### a. Practical Application by Physical Transformation

The examiner first shall review the claim and determine if it provides a transformation or reduction of an article to a different state or thing. If the examiner finds such a transformation or reduction, the examiner shall end the inquiry and find that the claim meets the statutory requirement of 35 U.S.C. § 101. If the examiner does not find such a transformation or reduction, the examiner has not determined as a final matter that the claim is non-statutory. The examiner must proceed in further inquiry.

b. Practical Application That Produces a Useful, Concrete, and Tangible Result

For eligibility analysis, physical transformation "is not an invariable requirement, but merely one example of how a mathematical algorithm [or law of nature] may bring about a useful application." *AT&T*, 172 F.3d at 1358-59, 50 USPQ2d at 1452. If the examiner determines that the claim does not entail the transformation of an article, then the examiner shall review the claim to determine if the claim provides a practical application that *produces* a useful, tangible and concrete *result*. In determining whether the claim is for a "practical application," the focus is not on whether the steps taken to achieve a particular result are useful, tangible and concrete, but rather that *the final result achieved* by the claimed invention is "useful, tangible and concrete." The claim must be examined to see if it includes anything more than a § 101 judicial exception. If the claim is directed to a practical application of the § 101 judicial exception producing a result tied to the physical world that does not preempt the judicial exception, then the claim meets the statutory requirement of 35 U.S.C. § 101. If the examiner does not find such a practical application, the examiner has determined that the claim is nonstatutory.

In determining whether a claim provides a practical application that produces a useful, tangible, and concrete result, the examiner should consider and weigh the following factors:

(1) "USEFUL RESULT"

For an invention to be "useful" it must satisfy the utility requirement of section 101. The USPTO's official interpretation of the utility requirement provides that the utility of an invention has to be (i) specific, (ii) substantial *and* (iii) credible. MPEP § 2107 and [*In re*] *Fisher*, 421 F.3d [1365,] ___, 76 USPQ2d [1225,] 1230 [(Fed. Cir. 2005)] (citing the Util-

ity Guidelines with approval for interpretation of "specific" and "substantial"). In addition, when the examiner has reason to believe that the claim is not for a practical application that produces a useful result, the claim should be rejected, thus requiring the applicant to distinguish the claim from the three § 101 judicial exceptions to patentable subject matter by specifically reciting in the claim the practical application. In such cases, statements in the specification describing a practical application may not be sufficient to satisfy the requirements for section 101 with respect to the claimed invention. Likewise, a claim that can be read so broadly as to include statutory and nonstatutory subject matter must be amended to limit the claim to a practical application. In other words, if the specification discloses a practical application of a § 101 judicial exception, but the claim is broader than the disclosure such that it does not require a practical application, then the claim must be rejected.

(2) "TANGIBLE RESULT"

The tangible requirement does not necessarily mean that a claim must either be tied to a particular machine or apparatus or must operate to change articles or materials to a different state or thing. However, the tangible requirement does require that the claim must recite more than a § 101 judicial exception, in that the process claim must set forth a practical application of that § 101 judicial exception to produce a real-world result. *Benson*, 409 U.S. at 71-72, 175 USPQ at 676-77 (invention ineligible because had "no substantial practical application."). "[A]n *application* of a law of nature or mathematical formula to a . . . process may well be deserving of patent protection." *Diehr*, 450 U.S. at 187, 209 USPQ at 8 (emphasis added); *see also Corning*, 56 U.S. (15 How.) at 268, 14 L.Ed. 683 ("It is for the discovery or invention of some practical method or means of producing a beneficial result or effect, that a patent is granted . . ."). In other words, the opposite meaning of "tangible" is "abstract."

(3) "CONCRETE RESULT"

Another consideration is whether the invention produces a "concrete" result. Usually, this question arises when a result cannot be assured. In other words, the process must have a result that can be substantially repeatable or the process must substantially produce the same result again. *In re Swartz*, 232 F.3d 862, 864, 56 USPQ2d 1703, 1704 (Fed. Cir. 2000) (where asserted result produced by the claimed invention is "irreproducible" claim should be rejected under section 101). The opposite of "concrete" is unrepeatable or unpredictable. Resolving this question is dependent on the level of skill in the art. For example, if the claimed invention is for a process which requires a particular skill, to determine whether that process is substantially repeatable will necessarily require a determination of the level of skill of the ordinary artisan in that field. An appropriate rejection under 35 U.S.C. § 101 should be accompanied by a lack of enablement rejection under 35 U.S.C. § 112, paragraph 1, where the invention cannot operate as intended without undue experimentation. *See infra.*

### 3. Determine Whether the Claimed Invention Preempts an Abstract Idea, Law of Nature, or Natural Phenomenon (§ 101 Judicial Exceptions)

Even when a claim applies a mathematical formula, for example, as part of a seemingly patentable process, the examiner must ensure that it does not in reality "seek[] patent protection for that formula in the abstract." *Diehr*, 450 U.S. at 191, 209 USPQ at 10. "Phenomena of nature, though just discovered, mental processes, abstract intellectual concepts are not patentable, as they are the basic tools of scientific and technological work." *Benson*, 409 U.S. at 67, 175 USPQ at 675. One may not patent a process that comprises every "substantial practical application" of an abstract idea, because such a patent "in practical effect would be a patent on the [abstract idea] itself." *Benson*, 409 U.S. at 71-72, 175

USPQ at 676; *cf. Diehr*, 450 U.S. at 187, 209 USPQ at 8 (stressing that the patent applicants in that case did "not seek to pre-empt the use of [an] equation," but instead sought only to "foreclose from others the use of that equation in conjunction with all of the other steps in their claimed process"). "To hold otherwise would allow a competent draftsman to evade the recognized limitations on the type of subject matter eligible for patent protection." *Diehr*, 450 U.S. at 192, 209 USPQ at 10. Thus, a claim that recites a computer that solely calculates a mathematical formula (*see Benson*) or a computer disk that solely stores a mathematical formula is not directed to the type of subject matter eligible for patent protection. If an examiner determines that the claimed invention preempts a § 101 judicial exception, the examiner must identify the abstraction, law of nature, or natural phenomenon and explain why the claim covers every substantial practical application thereof.

**D. Establish on the Record a Prima Facie Case**

The examiner should review the totality of the evidence (e.g., the specification, claims, relevant prior art) before reaching a conclusion with regard to whether the claimed invention sets forth patent eligible subject matter. The examiner must weigh the determinations made above to reach a conclusion as to whether it is more likely than not that the claimed invention as a whole either falls outside of one of the enumerated statutory classes or within one of the exceptions to statutory subject matter. "The examiner bears the initial burden . . . of presenting a *prima facie* case of unpatentability." *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). If the record as a whole suggests that it is more likely than not that the claimed invention would be considered a practical application of an abstract idea, natural phenomenon, or law of nature, the examiner should not reject the claim.

After the examiner identifies and explains in the record the basis for why a claim is for an abstract idea with no practical application, then the burden shifts to the applicant to either amend the claim or make a showing of why the claim is eligible for patent protection. *See, e.g., In re Brana*, 51 F.3d 1560, 1566, 34 USPQ2d 1436, 1441 (Fed. Cir. 1995); *see generally* MPEP § 2107 (Utility Guidelines).

* * * * *

Date: 10/26/05

/s/
JOHN J. DOLL
Commissioner for Patents

## ANNEX I

### Flowchart for Subject Matter Eligibility

* * * *

**DETERMINE WHETHER THE CLAIMED INVENTION COMPLIES WITH THE SUBJECT MATTER ELIGIBILITY REQUIREMENT OF 35 U.S.C. § 101**

- Does the Claimed Invention Fall Within an Enumerated Statutory Category?
- Does the Claimed Invention Fall Within a § 101 Judicial Exception—Law of Nature, Natural Phenomena or Abstract Idea?
    - Does the Claimed Invention Cover a § 101 Judicial Exception, or a Practical Application of a § 101 Judicial Exception?
        - Practical Application by Physical Transformation?
        - Practical Application That Produces a Useful (35 U.S.C. § 101 utility), Tangible, and Concrete Result?
    - Does the Claimed Invention Preempt an Abstract Idea, Law of Nature, or Natural Phenomenon (§ 101 Judicial Exception)?
- Establish on the Record a Prima Facie Case

* * * *

## ANNEX III

### Improper Tests For Subject Matter Eligibility

As set forth in the patent eligible subject matter interim guidelines, a practical application of a 35 U.S.C. § 101 judicial exception is claimed if the claimed invention physically transforms an article or physical state to a different state or thing, or if the claimed invention otherwise produces a useful, concrete, and tangible result. Therefore the following tests are ***not*** to be applied by examiners in determining whether the claimed invention is patent eligible subject matter:

(A) "not in the technological arts" test

(B) *Freeman-Walter-Abele* test

(C) mental step or human step tests

(D) the machine implemented test

(E) the *per se* data transformation test.

* * * *

c. (i) The Mental Step Test

If a claimed process is performed by a machine, it is immaterial whether some or all the steps could be carried out by the human mind. As stated in [*In re*] *Musgrave*, 431 F.2d [882,] 893, 167 USPQ [280,] 289-90 [(C.C.P.A. 1970)]: "[W]e cannot agree with the board that these claims (all the steps of which can be carried out by the disclosed apparatus) are directed to non-statutory processes merely because **some or all** [emphasis added] the steps therein can also be carried out in or with the aid of the human mind or because it may be necessary for one performing the processes to think." Therefore, USPTO personnel should no longer rely on the mental step test to determine whether a claimed invention is directed to statutory subject matter. If all the steps of a claimed proc-

ess can be carried out in the human mind, examiners must determine whether the claimed process produces a useful, tangible, and concrete result, i.e., apply the practical application test set forth in *State Street*.

c. (ii) The Human Step Test

It is immaterial whether the process may be performed by some or all steps that are carried out by a human. Claims are not directed to non-statutory processes merely because **some or all** the steps therein can also be carried out in or with the aid of a human or because it may be necessary for one performing the processes to do some or all of the process steps. The inclusion in a patent of a process that may be performed by a person is not fatal to patentability. *Alco Standard Corp. v. Tennessee Valley Authority*, 808 F.2d 1490, 1496, 1 USPQ2d 1337, 1341 (Fed. Cir. 1987) (citing *Diehr*, 450 U.S. at 175); *see e.g. Smith & Nephew, Inc. v. Ethicon, Inc.*, 276 F.3d 1304, 61 USPQ2d 1065 (Fed. Cir. 2001) (method claim where all the steps are carried out by a human). Therefore, USPTO personnel should no longer rely on the human step test to determine whether a claimed invention is directed to statutory subject matter.

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